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IMPAIRMENT OF THE FLAVOR OF FISH BY WATER POLLUTANTS

Dean L. Shumway, et al

Oregon State University

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Impairment of the Flavor of Fish by Water Pollutants

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Estimated threshold concentrations were determined for twenty two organic compounds. The values ranged from 0.4 ppb (2,4-dichlorophenol) to 95 ppm (formaldehyde). An additional twelve compounds were tested, seven of which were not found to impair flavor at or near lethal levels.

Estimated threshold concentrations were determined for effluents from the Corvallis Sewage Treatment Plant, kraft paper mills, and a sulfite-base paper mill. The estimated threshold concentrations for primary, secondary, and secondary chlorinated effluents from the Corvallis plant were determined to be 11-13, 21-23, and 20-26 percent by volume, respectively. The estimated threshold concentrations for the effluents from the kraft and sulfite-base paper mills were about 6 and 36 percent by volume, respectively. ~~(Shumway 05D).~~ (EPA)

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IMPAIRMENT OF THE FLAVOR OF FISH BY WATER POLLUTANTS

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ABSTRACT

Laboratory studies with fish were conducted to determine an appropriate bioassay procedure for the examination of the flavor-imparting capacity of wastes and waste components (organic compounds). In addition, the flavor-imparting capacity and estimated threshold concentrations were determined for a number of organic compounds and effluents. Flavor evaluations were obtained through the use of taste panels.

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The estimated threshold concentrations for primary, secondary, and secondary chlorinated effluents from the Corvallis Sewage Treatment Plant were determined to be 11-13, 21-23, and 20-26 percent by volume, respectively.

This report was submitted in fullfillment of Project Number 18050 DDM under the (partial) sponsorship of the U.S. Environmental Protection Agency.

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SECTION I

CONCLUSIONS

(1) A standard bioassay procedure was developed and accepted for use in laboratory studies on the flavor-imparting capacity of wastes and waste components (organic compounds). The bioassay conditions selected are: dissolved oxygen - near air-saturation, light level-subdued or absent, pH - 7.0 to 8.0, temperature - 15°C for coldwater fish and 20-25°C for warmwater fish, exposure period - 48 hours, weight of fish per chamber - 150 to 1,000 grams in present apparatus, and exposure system - flowing water.

(2) The flavor of trout exposed to 2,4-dichlorophenol, butanethiol, o-cresol, and pyridine was found to reach maximum impairment in less than 33.5 hours. After exposure to 2,4-dichlorophenol for 24 hours, trout lost the acquired off-flavor in about 33.5 hours.

(3) The influence of exposure to concentrations of two flavor-imparting substances was examined. The observed off-flavor values were less than additive.

(4) Estimated threshold concentrations were determined for twenty two organic compounds. In addition, twelve organic compounds were tested for threshold concentrations, seven of which were not found to impair flavor at or very near lethal concentrations.

(5) Estimated threshold concentrations were determined for effluents from two kraft process paper mills and a sulfite-base paper mill. The estimated threshold concentration for the kraft effluents ranged from 5 to 8 percent by volume. The estimated threshold concentration of the sulfite effluent was 36 percent by volume.

(6) Primary, secondary, and secondary chlorinated effluents from the Corvallis Sewage Treatment Plant were evaluated. The estimated threshold concentration values ranged from 11 to 13 percent by volume for primary effluent, from 21 to 23 percent by volume for secondary effluent, and from 20 to 26 percent by volume for secondary chlorinated effluent.

SECTION II

RECOMMENDATIONS

(1) Fish are but one of a large number of important organisms that may be contaminated by the discharge of tainting substance into the aquatic environment. Studies should be initiated to determine the impact of such materials on the quality of other organisms such as crayfish, crabs, clams, oysters, lobsters, and other forms of edible aquatic life, both fresh water and marine.

(2) With the methods employed in this study it would be possible to determine the flavor-imparting capacity of a large number of different types of domestic and industrial effluent. Such information would be extremely valuable to state and federal agencies responsible for establishing water quality and effluent standards.

(3) This program was limited to a preliminary examination of treated waste water collected from one source (Corvallis). Effluents from other treatment plants of the same general kind as well as other kinds should be examined for their flavor-imparting capacities.

(4) Since many effluents contain numerous components capable of impairing the flavor of fish, the interaction of combinations of these compounds should receive further detailed study.

(5) More sophisticated and less laborious procedures should be developed for the examination of tissue for impaired flavor. Taste panels, although adequate for many needs, can neither determine concentration of substances causing off-flavors nor examine small samples of flesh. When taste panels are employed, at least fifteen trained judges should be employed. Panels comprised of fewer than ten judges probably should be avoided.

(6) Information is needed on the relationship between the exposure concentration of a compound and the resulting tissue concentration and degree of flavor impairment.

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SECTION III

INTRODUCTION

The value of many recreationally and commercially important fisheries is constantly being reduced by the introduction of fish-tainting substances into surface waters. While considerable effort has been directed toward the sensory evaluation of foods and drinking water in recent years, only a limited effort has been expended in determining the flavor-imparting capacity of wastes and waste components (organic compounds) commonly entering lakes and streams.

In only a few cases have the source and nature of substances causing tainted fish problems been accurately identified (Westfall and Ellis, 1944; Tamura, Itazawa, and Morita, 1954; Böetius, 1954; Fetterolf, 1964; Hasselrot, 1964; Nitta, *et al.*, 1965; Shumway, 1966, Krishnaswami and Kupchanko, 1969). Wastes discharged from kraft paper plants, chemical plants producing pesticides, coal-tar processing, and oil refineries have been identified, or strongly suggested, as sources of fish tainting. Unfortunately, very little is known about the flavor-imparting capacity of the waste components (specific organic compounds) comprising these wastes.

Results of studies on the flavor-imparting capacity of wastes or waste components (organic compounds) have been reported by Albersmeyer and Erichsen (1959), Brandt (1955), Böetius (1954), Schultz (1961), Shumway (1966), Shumway and Chadwick (1971), and Winston (1959). These investigations, conducted under controlled laboratory conditions, dealt mainly with phenolic compounds, although Shumway and Chadwick (1971) studied treated and untreated kraft mill effluents.

A wide variety of organic compounds are capable of imparting objectionable tastes and odors to the flesh of fish and other aquatic organisms. In many cases these materials are capable of impairing flavor at concentrations far below levels otherwise considered detrimental to aquatic organisms. To adequately protect our freshwater fisheries, both commercial and sport, we must not only ensure that reproduction, growth, migration, and other essential activities of fish will be protected, but also ensure that the flavor of the flesh of fish will not be impaired beyond acceptable limits. Without this protection, otherwise productive fish populations may become largely unutilized by man. With this in mind, the research covered in this final report was planned and conducted.

Presented in this report are the results of a three-year laboratory study on the influence of wastes and specific organic compounds on the flavor of the flesh of freshwater fish. There were two objectives to the three-year study. (1) To develop, through studies with freshwater fish held in flowing water, appropriate standard procedures for the evaluation of the flavor-imparting capacity of contaminants. The

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standardization of procedures was to include selection of specific conditions for exposure of the fish and of methods of organoleptic evaluation of the flesh. (2) To determine through studies using freshwater fish held in flowing water the flavor imparting capacity of a substantial number of wastes and organic compounds commonly discharged into surface waters. The research was conducted at the Oak Creek Fisheries Laboratory of the Department of Fisheries and Wildlife, Oregon State University, during the period of April 1, 1969 to March 31, 1972.

SECTION IV

FISH FACILITIES

The design of the study and number of fish required necessitated the construction of facilities at which relatively large numbers of fish of several species could be held and reared. Facilities suitable for the rearing of trout were constructed at the Averill Fisheries Laboratory located east of Corvallis. Facilities for holding warmwater fish and facilities for temporary retention of trout were constructed at the Oak Creek Fisheries Laboratory located west of Corvallis.

Averill Facilities: The Averill Fisheries Laboratory was selected for holding and rearing trout primarily because it had a dependable and plentiful year-round supply of well-water, with temperatures ranging from 10°C in winter to 13°C in summer. In addition, problems with fish diseases were nearly unknown at the location, as well as at other laboratories located nearby.

Ten circular, self-cleaning, fish-holding tanks and one oval starter-tank were assembled at the Averill site. The circular tanks were 2 ft. deep and ranged in diameter from 5 to 8 ft. The oval tank was 1-ft. deep, 2 ft. wide and 8 ft. long. The tanks, made of galvenized metal, were originally intended as stock-watering tanks. Prior to installation, the tanks were sandblasted and painted with epoxy paint. Each tank was fitted with a self-cleaning standpipe system, metal-screen or nylon-mesh covers to prevent fish from jumping out, and a water delivery system. Five tanks were supplied with automatic feeding devices. A small metal building, constructed on the site, was furnished with a work table, sink, and freezer (storage of fish food) and has storage space for maintenance supplies and tools. A 6-ft., metal-link fence protects the fish and facilities from vandalism and unauthorized entry. A photograph of the Averill site is presented in Figure 1.

Oak Creek Facilities: A second facility, capable of handling fish collected from the wild or obtained from state owned hatcheries, was constructed at the Oak Creek Fisheries Laboratory. This facility also allowed the short-term retention of a limited number of fish reared at the Averill site, thus facilitating fish handling requirements. Three circular tanks of the type described above were assembled at the Oak Creek site. Two 8-ft. tanks were used for warmwater fish and one 5-ft. tank was used for temporary holding of Averill fish. The tanks were covered with nylon-mesh nets and supplied with spring-water from the water supply of the Oak Creek Fisheries Laboratory.

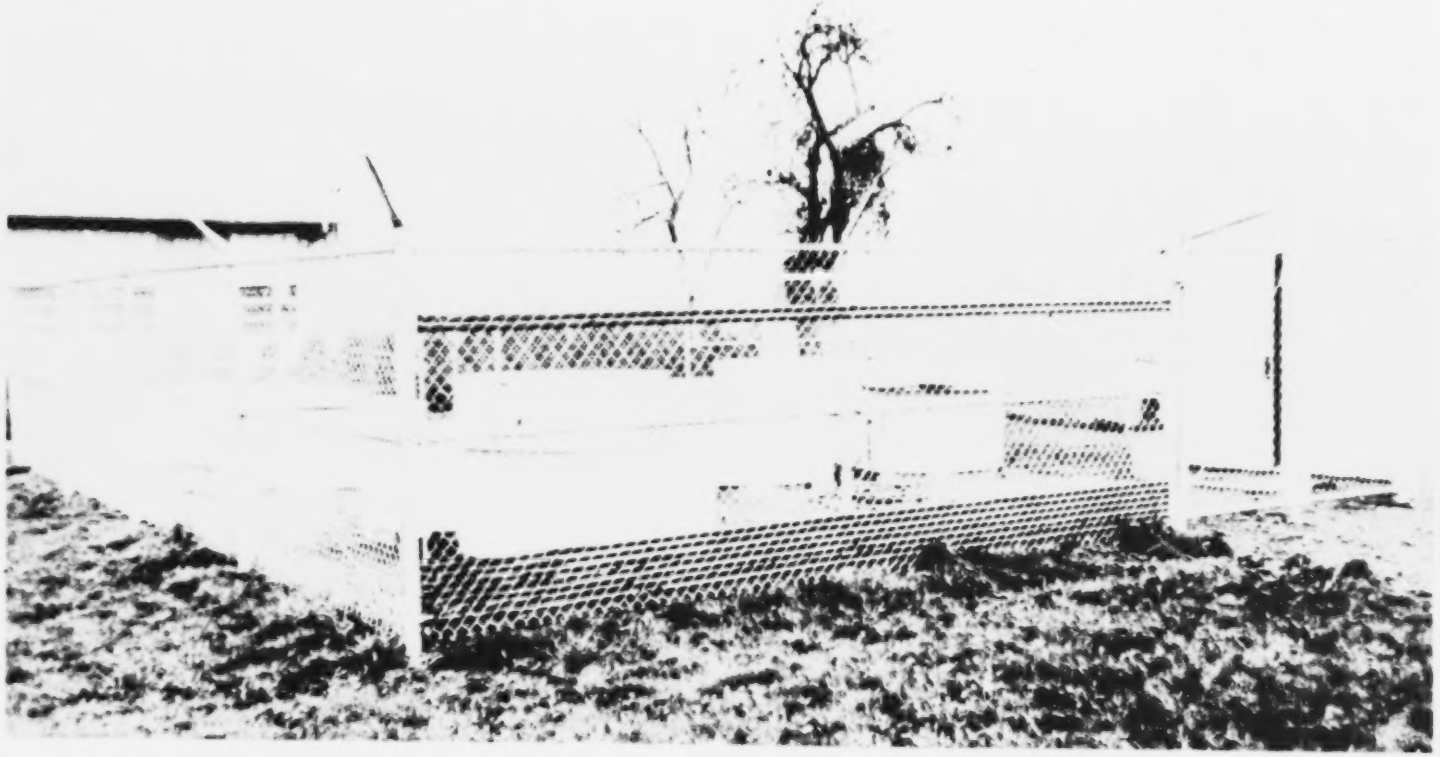

Figure 1. Photograph of the facilities used for rearing rainbow trout at the Averill Fisheries Laboratory located east of Corvallis.

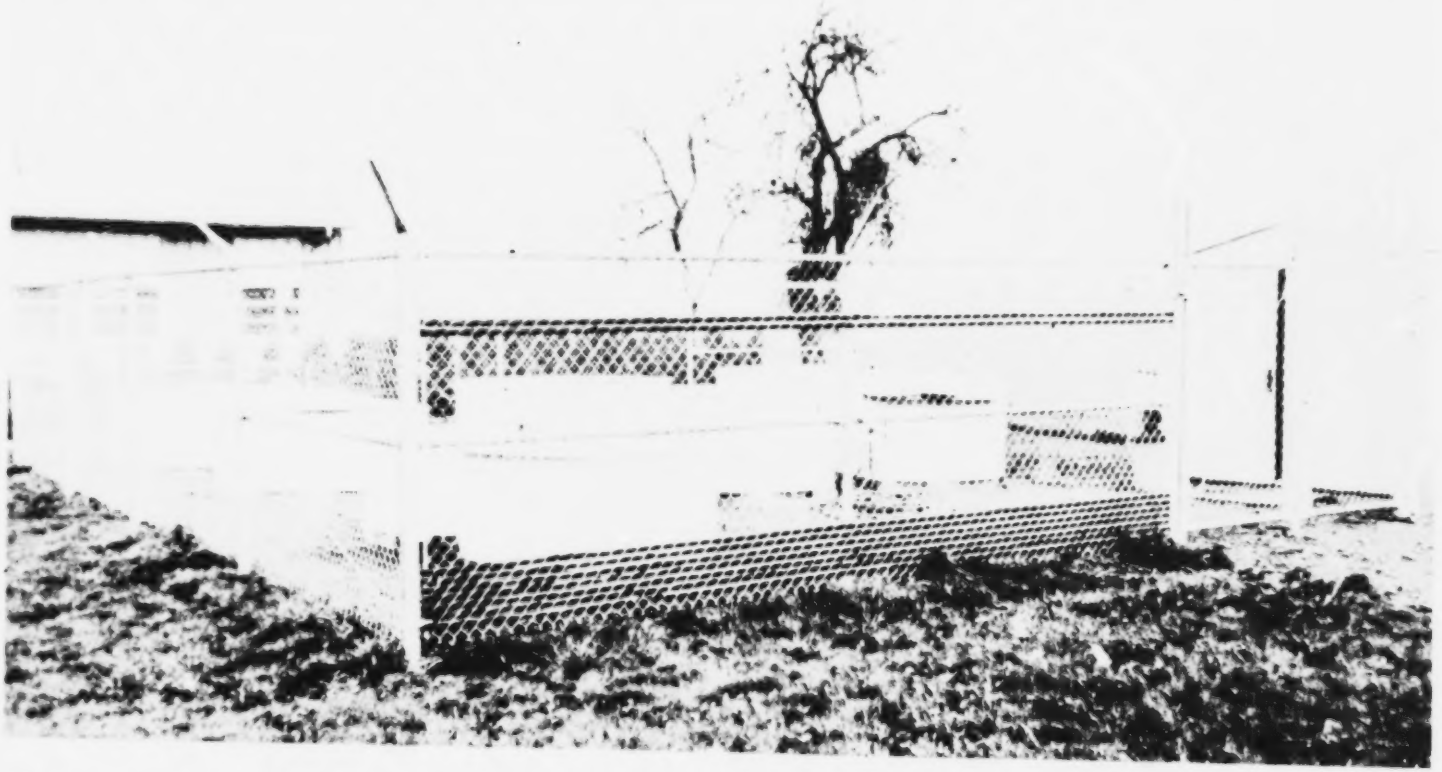

Figure 1. Photograph of the facilities used for rearing rainbow trout at the Averill Fisheries Laboratory located east of Corvallis.

SECTION V

MATERIALS, APPARATUS, AND METHODS

Experimental fish: Rainbow trout, *Salmo gairdneri*, largemouth bass, *Micropterus salmoides*, and bluegill, *Lepomis macrochirus*, were used as test fish in this investigation. Rainbow trout was the main test fish, with bass and bluegill used only to a limited extent.

Two stocks of rainbow trout were selected initially and brought to the Averill site. Kamloops trout (a variety of rainbow trout) eggs were obtained from the Trout Lodge Springs Hatchery located in eastern Washington. The second stock of trout was obtained as eggs from Dr. L. Donaldson's rainbow trout stock at the University of Washington. These stocks were selected for consideration as the eventual test trout because of their rapid growth characteristics and adaptability to hatchery operations. Reared under nearly identical conditions at the Averill site, the Kamloops trout were found to grow somewhat more rapidly than did Dr. Donaldson's variety; therefore, the Kamloops trout were selected as the test stock.

During most of the first year of the study, rainbow trout reared at our Averill site were too small for test purposes. During this stage of the study, it was necessary to use rainbow trout obtained from the Oregon State Game Commission's Roaring River Trout Hatchery located near Scio, Oregon. These fish were transported from the hatchery to the Oak Creek site in plastic-lined 55-gal. tanks. The hatchery trout were fed Oregon Moist Pellets, a product obtained from a local dealer, both at the hatchery and while being held at the Oak Creek Laboratory.

Since trout eggs and alevins could not be adequately handled at the Averill site, this was accomplished at an adjoining laboratory of the Research Division of the Oregon State Game Commission, where facilities were available. When ready to feed, the alevins were transported from the laboratory to the Averill site in buckets and reared to the desired size. The Oregon Moist Pellet was the only food provided the test animals. Although a few fish experienced a mild bacterial infection of the dorsal and caudal fins, no major disease problem developed during the course of the three-year study.

Largemouth bass and bluegill were used as test fish in a few experiments. The bass were obtained by seining from sloughs and ponds along the Willamette River. Bluegill were captured from local ponds by angling. After capture, the bass and bluegill were transported to the Oak Creek site in plastic-lined 55-gal. tanks and held until needed. Food in the form of live fish, angleworms, and Oregon Moist Pellets was provided the bass and bluegill.

Effluents tested: Kraft mill effluents (KME) were obtained from mills located near Halsey and Albany, Oregon. Both mills process pulp and paper from Douglas fir, *Pseudotsuga menziesii*, treat their effluents in large aerated stabilization lagoons and discharge treated effluents directly into the Willamette River. The Albany and Halsey mills process about 100 and 400 tons of pulp per day, respectively. The water use rate for the Albany mill is about 10,000 gal. per ton; 35,000 gal. of water per ton is required by the Halsey mill. The effluent discharged from the Albany mill has a BOD of about 50 to 75 ppm; the BOD in the Halsey mill effluents is normally less than 20 ppm.

Effluent was obtained from an ammonia-base, sulfite mill located near Lebanon, Oregon. The mill, which primarily produces liner board, processes about 100 tons of pulp daily and uses 40,000 gal. of water per ton. The effluent from this mill passes through an aerated stabilization lagoon before being discharged into the South Santiam River. BOD level of the effluent is normally at or below 125 ppm.

The Corvallis Sewage Treatment Plant was used as the source for primary, secondary, and secondary chlorinated waste water. The input to the Corvallis plant, which has a total capacity of about 15 million gallons per day (MGD) is nearly all from domestic sources. The plant employs a primary clarifier, two trickling filters, and a secondary clarifier. Samples of primary treated waste water were collected at a point between the primary clarifier and the trickling filters. Secondary treated waste was collected at the point of discharge from the trickling filters. In the Corvallis plant, chlorination of the effluent occurs between the trickling filter and the secondary clarifier. Secondary, chlorinated waste water was collected from the discharge of the secondary clarifier. The treated waste water from the Corvallis plant is discharged directly into a small creek a short distance above the confluence of the Willamette River.

During the time experiments were being conducted, the plant discharge rate and the BOD level of the inflow averaged 7.5 MGD and 168 ppm, respectively. The ranges in discharge rate and inflow BOD were 4.4 to 12.0 MGD and 43 to 295 ppm, respectively. No attempt was made to determine residual chlorine levels of the treated effluent after chlorination and discharge from the secondary clarifier. Toxicity data gathered during the tests strongly suggest that the chlorine level fluctuated substantially and was probably quite high most of the time.

Chemicals tested: With only a few exceptions, the chemicals used in this investigation were reagent grade. In the excepted cases, reagent grade chemicals were not available and a chemical pure grade (exceeding U.S. Pharmacopoeia and National Formulary) was utilized. All chemicals used were obtained from either the J. T. Baker Chemical Company or the Mallinckrodt Chemical Company.

Experimental apparatus: The three experimental apparatus used in this study were located in a 15°C constant temperature room which was lighted continually with fluorescent lights. Figure 2 is a schematic drawing of one of the three independently controlled, dilution apparatus. One diluter supplied well-water to six exposure chambers; the other two diluters supplied well-water to four chambers each. The flow of water entered the 70-liter fiberglass exposure chambers at a rate of 250 ml/min. In addition, each diluter was equipped with a temperature-control unit, an oxygen supply, and a toxicant delivery system. A Geological Survey analysis of the well-water from the source used in this study is presented in Appendix 1.

Well-water entered the diluter system via a water-control box constructed of wood, where it was continually circulated by a small submersible pump and brought to the desired temperature by a thermostatically controlled, stainless-steel immersion heater (Figure 2). From the control box, the water passed into a tubular, plastic, manifold and was meted into the glass mixing boxes through a small-diameter, adjustable delivery tube. Each mixing box was fitted with a simple, constant-head overflow tube and two adjustable delivery tubes. The larger of the two delivery tubes discharged water to the exposure chamber and the other delivery tube meted a small quantity of water into a lower mixing box. The flow of water through the delivery tubes could be stopped by rotating the discharge orifice into an up position. This was done with the delivery tube leading to the lowest mixing box, through which only control water was passed. Overflow water from the mixing boxes was discharged from the system.

Contaminant solutions were introduced to the dilution at the uppermost mixing box, where it was combined with an appropriate quantity of well-water and passed on through the system in the manner previously described. A simple, pivoting device was sometimes used at the point of contaminant introduction as a safety precaution. If the flow of exchange water slowed significantly or stopped, the safety device changed position and diverted the flow of contaminant away from the mixing box, thus preventing increases of concentration in the exposure chambers.

Each exposure chamber was provided with a cover to prevent fish from jumping out and to reduce the light level, a centrally located, stainless-steel standpipe for discharge of excess exchange water and for water-level control, a relief tube for removal of water samples, a mercury thermometer, inserted through the cover, and a water delivery port. The exposure chambers could be quickly and easily drained by removal of the standpipes.

Two types of contaminant control systems were employed. Twenty-liter Mariotte bottles (constant-head bottles) were used for the introduction of stock solutions of the organic compounds tested. Because of the large volume needed, sewage treatment plant and paper process effluents required a much more complicated delivery system. A 750-gal. fish holding tank was placed in the constant-temperature room near the

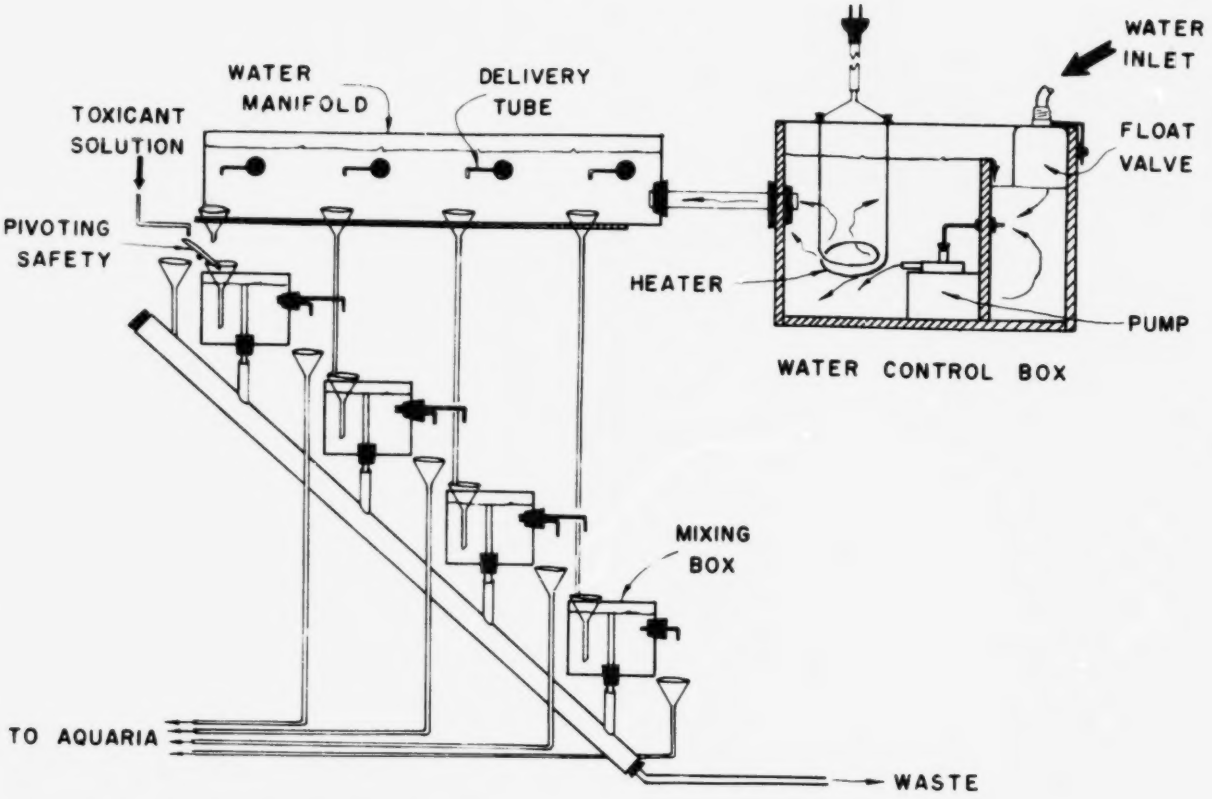

Figure 2. Drawing of the dilution apparatus used for the delivery of solutions to the exposure chambers.

exposure apparatus and filled with effluent. A small, submersible pump was placed on the tank and served to deliver a continuous supply of effluent to a wooden box position above the first mixing box of the largest diluter. The wooden box contained an overflow tube through which excess effluent returned to the holding tank and several delivery tubes similar to those described earlier. Through these tubes the effluent was meted to the diluter. The effluent was not aerated while in the large tank or wood head box.

Bottled gas, either air, oxygen, or an oxygen-nitrogen mixture was used to maintain the desired dissolved oxygen concentrations in the exposure chambers. The compressed gas passed from the gas cylinder through a two-stage reduction valve, a gas manifold, ball-displacement, gas flowmeters, and into the exposure chambers via gas dispersers. The introduction of gas also served to ensure thorough mixing of the renewal solution with that already present in that chamber. Although compressed air and pure oxygen were used in some experiments, the introduction of a mixture of 30% oxygen and 70% nitrogen proved highly satisfactory in maintaining oxygen concentration at or near air-saturation without excessive agitation of the water.

A photograph of one of the three exposure apparatus is present in Figure 3.

Experimental procedures: The day prior to the start of each experiment, the water and gas flows were adjusted to the desired levels and the contaminant stock solution or effluent prepared for introduction. During the first day of the experiment, the exposure chambers were drained of water, the contaminant flow started and adjusted, and the chambers filled. Test fish were then introduced, the number of fish per chamber depended on the experimental design, the size, and the species of fish used. Observations and necessary adjustments of water and contaminant flow rates and water temperature were made two to three times a day during the experiment. Dissolved oxygen concentrations were normally determined daily using the azide modification of the iodometric method; pH values varied little and were determined only periodically.

When an experiment was terminated, the test fish were removed from each chamber, killed, weighed and measured, cleaned (head and viscera removed), placed in labeled plastic bags, and either held under refrigeration at near 5°C for not more than 24 hours or frozen for a few days until they could be prepared for flavor evaluation. After the fish were removed, the flow of contaminant was stopped, the exposure chambers drained, and the diluter and exposure chambers cleaned with a mild detergent and thoroughly rinsed with acetone. Once cleaned, the diluter and exposure chambers were reassembled and the flow of water started once again.

The test fish were prepared for organoleptic evaluation by personnel of the Sensory Evaluation Section of the Department of Food Science and Technology, Oregon State University. The Sensory Evaluation Section,

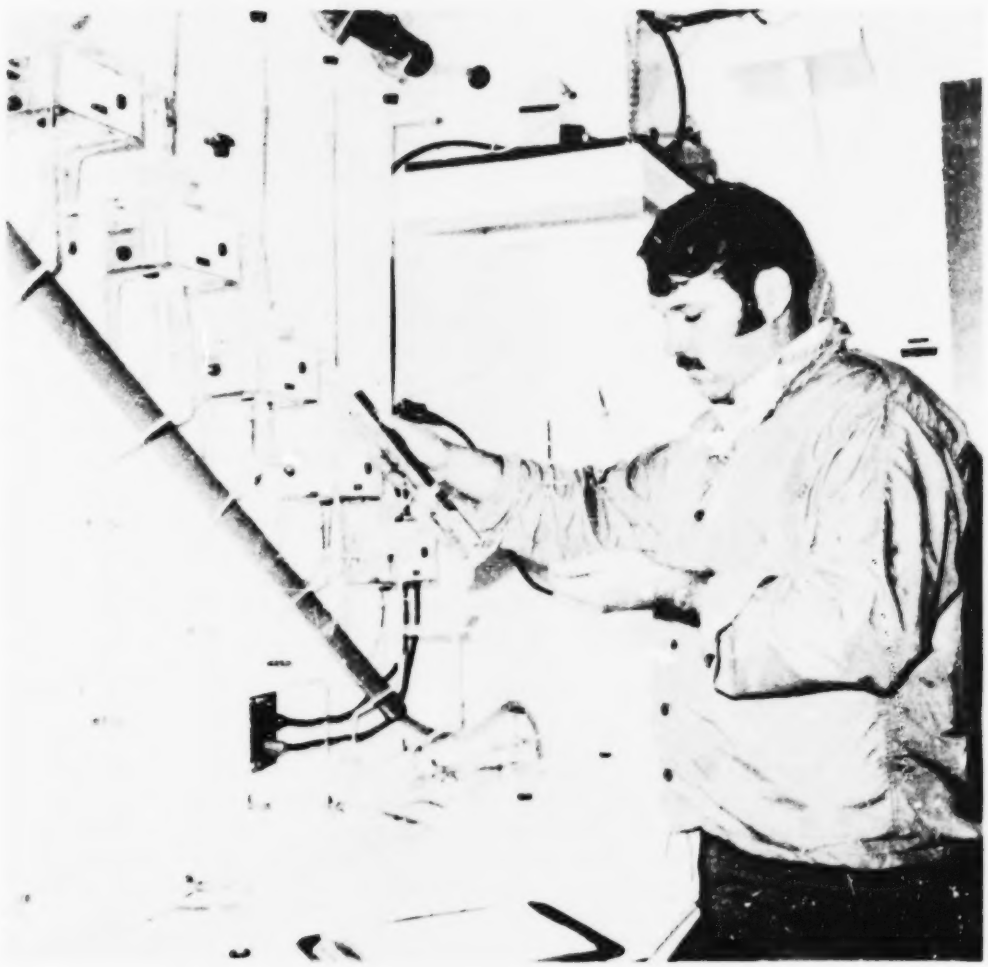

Figure 3. Photograph of the exposure apparatus showing the dilution system, water control box, and the covered exposure chambers.

under the direction of Mrs. Lois S. McGill, Professor of Food Science and Technology, also provided the facilities for the preparation and the evaluation of the samples of fish. Each sample of cleaned fish was wrapped in aluminum foil, placed in an individual pan, and cooked in an oven at approximately 210°C until done (approximately 30 to 40 min). No seasoning was added to the samples at any time. Each sample of fish was then removed from the oven, skinned and boned, the flesh of all fish within a sample lightly flaked and thoroughly mixed and a portion of flesh placed in each of a number of small, coded paper cups. The number of cups for each sample was determined by the number of judges that would evaluate that sample. In most experiments, the control, or uncontaminated sample of fish, was divided between coded cups and cups marked "reference." One cup from each group, including a cup from the group marked "reference," was placed on each of a number of small trays (one tray per judge). The tray of samples was then served immediately to the judge who was seated in an individual isolation booth (Figure 4).

The judges, all of whom had previous experience in evaluating the flavor of fish, were asked to smell the samples, and then to taste (masticate) and score the samples on a 7-point word evaluation scale for intensity of off-flavor (Appendix 2). They were asked to leave those samples with the most intense odor to be rated last. They were told that the sample marked "reference" contained flesh from the control fish. Judges were not required to tast samples with extremely intense or obnoxious odors. The word evaluation scale for off-flavor shown in Appendix 2 was converted to a number scale of 0 to 6, with 0 representing the highest quality (no off-flavor) and 6 the lowest quality (extreme off-flavor).

In addition to evaluating the sample of flesh for off-flavor, the judges were asked to rate each sample for overall desirability. The 7-point hedonic scale presented in Appendix 2 was used. The ratings ranged from very desirable (0) to very undesirable (6). The results of the hedonic ratings, when compared with the results of the off-flavor ratings, were found less instructive and are not included in this report.

Once the samples were evaluated, their ratings were compiled and the data treated statistically. A standard, two-way analysis of variance program (ANOVA) written for the CDC 3300 in FORTRAN language was used to test for experimental differences.

Near the completion of the first year, an attempt was made to develop an evaluation system that utilized "odor" rather than "taste." This was done because many judges strongly objected to the idea of masticating the foul-tasting fish flesh that often resulted from the exposure tests. In order to determine whether or not a change in procedure was advisable, comparative tests were made for a number of test compounds. In these tests, the judges were first asked to rate the samples on the basis of smell only, record their judgments, and then to re-evaluate the samples by the procedure normally utilized (tasting). The results showed that with some chemicals the two methods were equally satisfactory, but with

Figure 4. Photographs showing a tray containing samples of fish being served to judge seated in an isolation booth (upper picture) and a judge evaluating samples of fish (lower picture).

other chemicals, the "odor" method proved far less sensitive and the results more variable. Based on these findings, the "odor" method was abandoned from further use.

Panel judges were selected on the basis of willingness to serve, dependability, and of demonstrated ability to judge differences in the flavor of fish flesh. Many of the panel judges that were used had previous experience in evaluating the flavor of fish. This source of judges was not sufficient to meet the needs of the project, however, and a training program for new judges was initiated. Training sessions were conducted at which a number of potential judges were asked to evaluate a series of samples of contaminated fish flesh. The fish had been previously exposed to different concentrations of a chemical, usually 2,4-dichlorophenol, the response to which had already been determined. Prior to evaluating the samples, the group of potential judges were carefully explained the procedures they must follow in making their judgments. After each training session, the evaluations made by each judge were compared with the average responses of the group, as well as with the results from previous evaluations by experienced panel judges. Those demonstrating the ability to make reasonably reliable judgments were selected as judges. Normally, two or more training sessions were required of a potential judge prior to his selection as a new panel member.

To encourage participation by the judges in what was often a distasteful experience (tasting), each judge was given a candy bar and $1 after each series of flavor evaluations. The candy bar served as a mild stimulant to return and provided a means of removing undesirable odors or tastes from the mouth. The payment of $1 per test appeared to encourage future participation, particularly with judges coming from the student level.

SECTION VI

RESULTS AND INTERPRETATIONS

As pointed out earlier, the first objective of this study was to develop an appropriate standard procedure for the evaluation of the flavor-imparting capacity of water contaminants. Five organic compounds, o-cresol, 2,4-dichlorophenol, pyridine, n-butyl alcohol, and butanethiol, were selected for use during the initial methodology-development phase. During the initial phase, experiments were conducted to further understanding of the effects of various factors (exposure time, dissolved oxygen concentration, pH, light, size or weight of fish, weight of fish per chamber, rate of loss, etc.) on the flavor-imparting capacity of organic compounds. After completion of the methodology period and the selection of a standard procedure, routine testing of organic compounds and effluents could commence.

Initial exposure tests: The first series of experiments were conducted to determine the approximate flavor-imparting capacity of each of the five selected compounds. In these experiments rainbow trout were exposed to a wide range of concentrations (five levels and a control) for 96 hours at 15°C. The dissolved oxygen concentration was maintained near air-saturation with compressed air; the pH of the water was 7.8. The test fish were not shielded in any way from direct room illumination. The results of the tests are presented in Table 1.

Of the five compounds tested, four produced significant impairment of flavor (off-flavor) of the flesh at higher concentrations. Only n-butyl alcohol failed to produce impaired flavor in the test trout, even though high off-flavor index of 3.45 was obtained at the concentration of 100 ppm, the highest level tested. During the remainder of the initial phase of the investigation, only the four compounds that demonstrated strong flavor-imparting capabilities were utilized.

Rate of flavor impairment: One of the most crucial aspects of the initial phase of the study was to determine the exposure time required to produce maximum impairment of flavor. To evaluate this aspect, a series of experiments were conducted on the rate of uptake (flavor impairment) by trout exposed for various periods of time to o-cresol, 2,4-dichlorophenol, butanethiol, and pyridine. Trout were exposed to "high," "medium," and "low" concentrations of each chemical and a control for periods of 0.25, 1.27, 6.5, 33.5, and 168 hours (one week). The "high" concentrations were well above previously estimated threshold concentrations (level at which flavor impairment begins to be detected), the "medium" concentrations were close to the estimated threshold concentrations, and the "low" concentrations were well below those levels. At the end of each test period, trout were removed from each concentration and the control chamber and frozen for flavor evaluation at a later date. The samples were evaluated (tasted) within a week after the last

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Table 1. Experimental conditions and results of tests in which trout were exposed to various concentrations of chemicals for 96 hours at 15°C.

Exper. no.	Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index[1/] (0-6)	$LSD_{.05}$[2/]	Standard error of the mean	Fish per chamber grams	Fish per chamber number
A-1	Butanethiol	August 28, 1969	Trout	10 Judgments			
	0		2.05	0.85	0.62	373	2
	1		2.45		0.40	473	2
	11		2.25		0.40	625	2
	114		1.85		0.45	466	2
	1,050		4.55	*	0.38	526	2
	11,150		5.25	*	0.28	587	2
A-2	Butanethiol	September 4, 1969	Trout	10 Judgments			
	0		1.45	0.96	0.29	492	2
	0.08		2.30		0.54	595	2
	0.8		2.55	*	0.54	504	2
	7.9		1.95		0.36	728	2
	79		3.70	*	0.41	547	2
	832		5.20	*	0.29	482	2
A-3	n-Butyl alcohol	July 31, 1969	Trout	10 Judgments			
		0	1.60	n.s.	0.36	-	2
		0.01	1.85		0.35	-	2
		0.1	2.00		0.30	-	2
		1	1.80		0.48	-	2
		10	2.10		0.51	-	2
		100	3.45		0.58	-	2
A-4	o-Cresol	August 13, 1969	Trout	10 Judgments			
		0	1.45	0.99	0.32	507	2
		0.001	1.80		0.43	560	2
		0.01	1.70		0.46	344	2
		0.1	1.35		0.25	449	2
		1	3.30	*	0.50	440	2
		10	4.00	*	0.44	470	2
		100	+		-	-	-
A-5	2,4-Dichlorophenol	July 10, 1969	Trout	10 Judgments			
	0		0.70	0.84	0.15	-	2
	0.01		1.25		0.25	-	2
	0.1		1.55	*	0.44	-	2
	1		1.75	*	0.24	-	2
	10		2.60	*	0.34	-	2
	100		4.40	*	0.34	-	2

Table 1. Continued

Exper. no.	Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index[1/] (0-6)	LSD[2/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number
A-6 Pyridine	July 24, 1969		Trout	10 Judgments			
	0		1.50	n.s.	0.32	338	2
	0.1		2.05		0.42	304	2
	1		2.20		0.44	398	2
	10		1.55		0.34	331	2
	100		2.20		0.47	309	2
	1,000		2.45		0.64	314	2
A-7 Pyridine	August 7, 1969		Trout	10 Judgments			
		0	1.75	0.78	0.45	486	2
		0.01	2.20		0.45	473	2
		0.1	2.20		0.37	376	2
		1	1.50		0.26	381	2
		10	1.55		0.31	381	2
		100	4.20	*	0.44	370	2

[1/] Off-flavor index based on a scale of 0 (no off-flavor) to 6 (very extreme off-flavor).

[2/] The least significant difference at P=0.05 based on a two-way analysis of variance. An asterisk (*) indicates a statistically significant change in flavor from that of the control sample.

samples were removed (168 hours).

Experimental conditions and results of the series of tests on the rate of flavor impairment are presented in Table 2. Trout exposed to the "high" concentration of each of the chemicals tested appeared to attain maximum off-flavor in 33.5 hours or less. Additional exposure time did not alter significantly the observed mean off-flavor indices. At the "medium" and "low" concentrations of each of the test chemicals, exposure time did not appear to influence the degree of flavor impairment.

The results of experiments with butanethiol (U-1, U-2, and U-3) and 2,4-dichlorophenol (U-7, U-8, and U-9) are presented graphically in Figure 5. As may be seen, rather substantial increases in off-flavor occurred at the "high" concentration of each chemical after exposure for only fifteen minutes (0.25 hour). Continued exposure for 1.27 hours at the "high" concentration of both compounds resulted in the attainment of maximum or near maximum flavor impairment. Additional exposure time caused little or no change in the degree of flavor impairment. At 10 and 1 ppb of butanethiol, the mean off-flavor index appeared to increase with exposure time, although the differences were not significant (Table 2).

Based on the results of the exposure-time tests, a standard exposure period of 48 hours was adopted. It may well be that longer exposure periods might produce a slightly higher degree of off-flavor; however, our data strongly suggest that if this is so the exposure period required would be longer than a week (168 hours), the maximum exposure period used in our tests. Long-term exposure periods would surely cause many additional testing problems (i.e., increased mortality, need to provide food, gear failures, disease, etc.).

Clearing rate: A series of experiments were conducted to determine the relation between the degree of off-flavor and the length of time held in fresh water after a 24-hour exposure to 100, 10, and 1 ppb of 2,4-dichlorophenol. Samples of trout were removed from each group after 0.25, 1.27, 6.5, 33.5, and 168 hours exposure to fresh water and frozen for later evaluation.

The results of the experiments on clearing rate are presented in Table 3 and Figure 6. Off-flavor values for trout exposed to 100 ppb of 2,4-dichlorophenol were substantially reduced after 6.5 hours in fresh water. After 33.5 hours in fresh water the flavor of the trout exposed at the 100 ppb level had returned to normal. Some reduction in off-flavor appeared to occur in the first hour at 10 ppb of 2,4-dichlorophenol, and complete loss had occurred after 6.5 hours of contact with fresh water. At the lowest level tested (1 ppb), little or no change occurred in the off-flavor index with exposure to fresh water. The changes in off-flavor values observed at the 10 and 1 ppb levels were not statistically significant (Table 2).

Table 2. The experimental conditions and results of tests in which rainbow trout were exposed at 15°C for various periods of time to high, intermediate, and low concentrations of pyridine, butanethiol, 2,4-dichlorophenol, and o-cresol.

Exper. no.	Concentration ppb	Concentration ppm	Exposure period (hrs.)	Mean off-flavor index[1/] (0-6)	LSD[2/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Dissolved oxygen (mg/l)	pH
U-1	Butanethiol	February 11, 1970		10 Judgments						
	0		0.25	1.30	n.s.	0.26	145	1	6.8	7.8
	1		0.25	1.05		0.14	110	1	9.8	7.8
	1		1.27	1.55		0.23	97	1	9.8	7.8
	1		6.5	1.80		0.28	153	1	9.8	7.8
	1		33.5	1.95		0.28	196	1	8.7	7.7
	1		168	1.80		0.47	170	1	8.7	7.7
U-2	Butanethiol	February 11, 1970		10 Judgments						
	0		1.27	1.15	n.s.	0.21	188	1	8.8	7.8
	10		0.25	1.45		0.39	191	1	8.6	7.8
	10		1.27	1.80		0.45	158	1	8.6	7.8
	10		6.5	2.20		0.34	192	1	8.6	7.8
	10		33.5	2.65		0.48	200	1	11.0	8.0
	10		168	1.90		0.26	114	1	11.0	8.0
U-3	Butanethiol	February 11, 1970		10 Judgments						
	0		33.5	1.35	1.04	0.26	164	1	8.8	7.8
	100		0.25	2.40	*	0.46	143	1	9.0	7.8
	100		1.27	3.60	*	0.43	140	1	9.0	7.8
	100		6.5	3.45	*	0.46	161	1	9.0	7.8
	100		33.5	3.65	*	0.45	131	1	8.9	7.8
	100		168	4.25	*	0.37	165	1	8.9	7.8

Table 2. Continued

Exper. no.	Concentration ppb	Concentration ppm	Exposure period (hrs.)	Mean off-flavor index[1/] (0-6)	LSD[2/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Dissolved oxygen (mg/1)	pH
U-4 O-Cresol	December 3, 1969	10 Judgments								
	0		1.29	1.15	n.s.	0.14	306	2	12.5	-
	12.5		0.25	0.93		0.15	471	4	10.6	-
	9.9		1.27	1.23		0.15	291	2	10.0	-
	12.5		6.5	1.35		0.26	243	2	10.6	-
	10.0		33.5	1.33		0.20	248	2	10.0	-
	9.6		168	1.70		0.30	251	2	10.6	-
U-5 O-Cresol	December 3, 1969	10 Judgments								
	0		6.5	1.03	n.s.	0.17	275	2	9.3	-
	110		0.25	1.62		0.23	490	4	10.4	-
	129		1.27	1.50		0.18	274	2	13.0	-
	110		6.5	1.43		0.31	252	2	10.4	-
	130		33.5	1.65		0.24	257	2	13.0	-
	124		168	1.55		0.29	256	2	10.4	-
U-6 O-Cresol	December 3, 1969	10 Judgments								
	0		168	1.18	n.s.	0.20	443	4	6.9	-
	826		0.25	2.45		0.27	495	4	8.0	-
	996		1.27	2.93		0.24	236	2	13.0	-
	826		6.5	2.60		0.40	260	2	8.0	-
	990		33.5	2.73		0.30	268	2	13.0	-
	958		168	2.78		0.33	235	2	8.0	-

Table 2. Continued

Exper. no.	Concentration ppb	Concentration ppm	Exposure period (hrs.)	Mean off-flavor index[1] (0-6)	LSD[2] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Dissolved oxygen (mg/l)	pH
U-7	2,4-Dichlorophenol		January 8, 1970	10 Judgments						
	0		0.25	1.58	n.s.	0.24	255	1	8.0	-
	0.1		0.25	1.03		0.14	246	1	5.7	-
	0.1		1.27	1.28		0.18	440	2	8.9	-
	0.1		6.5	1.25		0.16	212	1	8.9	-
	0.1		33.5	1.53		0.27	453	2	8.8	-
	0.1		168	1.45		0.22	239	1	9.8	-
U-8	2,4-Dichlorophenol		January 8, 1970	10 Judgments						
	0		1.27	1.38	n.s.	0.19	393	2	8.0	-
	10		0.25	2.20		0.22	193	1	6.3	-
	10		1.27	1.80		0.23	367	2	7.8	-
	10		6.5	1.95		0.17	213	1	7.8	-
	10		33.5	2.20		0.29	368	2	9.2	-
	10		168	1.85		0.21	188	1	9.7	-
U-9	2,4-Dichlorophenol		January 8, 1970	10 Judgments						
	0		168	1.33	0.62	0.22	222	1	8.8	-
	100		0.25	2.65	*	0.21	192	1	7.0	-
	100		1.27	3.83	*	0.29	446	2	7.7	-
	100		6.5	3.93	*	0.18	179	1	7.7	-
	100		33.5	3.30	*	0.23	381	2	7.6	-
	100		168	3.50	*	0.28	183	1	9.4	-

Table 2. Continued

Exper. no.	Concentration ppb	Concentration ppm	Exposure period (hrs.)	Mean off-flavor index[1/] (0-6)	LSD[2/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Dissolved oxygen (mg/l)	pH
U-10	Pyridine	January 22, 1970		10 Judgments						
		0	6.5	0.85	n.s.	0.15	238	1	11.0	7.9
		1	0.25	0.95		0.23	638	3	10.3	8.0
		1	1.27	1.35		0.36	390	2	10.3	8.0
		1	6.5	1.00		0.21	173	1	10.3	8.0
		1	33.5	1.40		0.29	348	2	8.8	8.0
		1	168	1.50		0.40	176	1	9.1	7.8
U-11	Pyridine	January 22, 1970		10 Judgments						
		0	33.5	0.95	n.s.	0.20	152	1	11.0	7.9
		10	0.25	1.05		0.32	571	3	9.8	7.9
		10	1.27	0.75		0.24	400	2	9.8	7.9
		10	6.5	1.25		0.24	212	1	9.8	7.9
		10	33.5	1.45		0.29	462	2	9.9	7.8
		10	168	1.45		0.36	265	1	9.2	7.8
U-12	Pyridine	January 22, 1970		10 Judgments						
		0	168	1.00	n.s.	0.18	189	1	10.3	7.9
		100	0.25	2.30		0.75	704	3	9.9	7.9
		100	1.27	2.60		0.70	442	2	9.9	7.9
		100	6.5	3.30		0.41	212	1	9.9	7.9
		100	33.5	3.80		0.33	391	2	8.9	7.9
		100	168	3.25		0.60	208	1	9.2	7.9

Table 2. Continued

Exper. no.	Concentration ppb	Concentration ppm	Exposure period (hrs.)	Mean off-flavor index[1] (0-6)	LSD[2] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Dissolved oxygen (mg/l)	pH
U-13	2,4-Dichlorophenol		October 6, 1970	9 Judgments						
	0		168	0.61	n.s.	0.25	335	1	-	-
	1		0.25	1.11		0.26	322	1	-	-
	1		1.27	1.61		0.30	350	1	-	-
	1		6.5	2.00		0.33	348	1	9.4	-
	1		33.5	1.05		0.32	355	1	9.0	-
	1		168	0.94		0.24	340	1	9.4	-
U-14	2,4-Dichlorophenol		October 6, 1970	10 Judgments						
	0		6.5	1.05	0.84	0.30	361	1	-	-
	10		0.25	0.85		0.24	355	1	-	-
	10		1.27	2.45	*	0.31	470	1	-	-
	10		6.5	1.50		0.26	372	1	8.0	-
	10		33.5	2.35	*	0.42	360	1	8.3	-
	10		168	1.20		0.42	302	1	9.6	-
U-15	2,4-Dichlorophenol		October 6, 1970	10 Judgments						
	0		0.25	0.45	0.79	0.17	342	1	-	-
	100		.25	1.45	*	0.24	316	1	-	-
	100		1.27	2.50	*	0.41	465	1	-	-
	100		6.5	2.70	*	0.44	449	1	8.0	-
	100		33.5	1.50	*	0.31	323	1	8.4	-
	100		168	1.65	*	0.17	299	1	9.6	-

[1] Off-flavor index based on a scale of 0 (no off-flavor) to 6 (very extreme off-flavor).

[2] The least significant difference at P=0.05 based on a two-way analysis of variance. An asterisk (*) indicates a statistically significant change in flavor from that of the control sample.

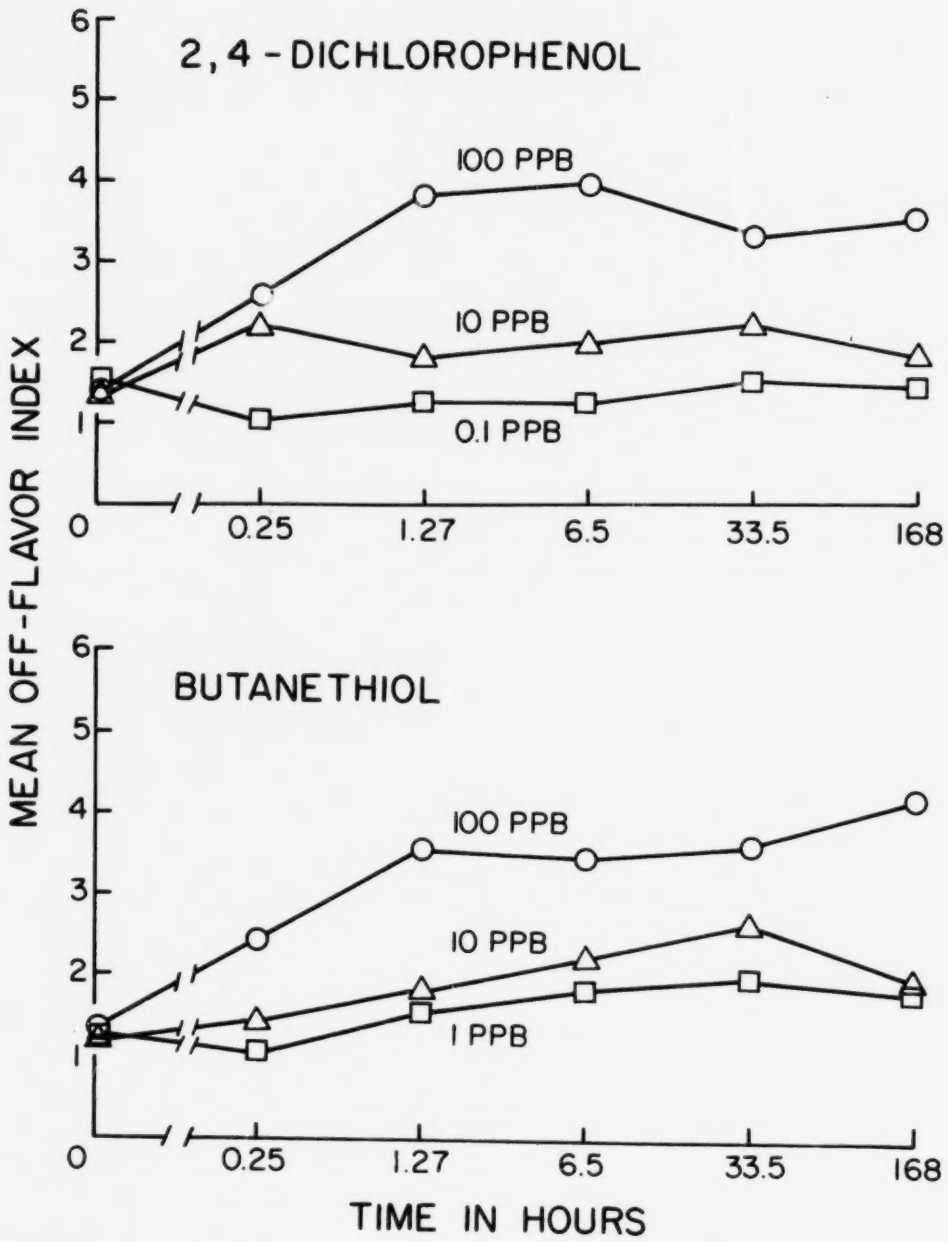

Figure 5. The influence of exposure time on the mean off-flavor indices for trout exposed to three concentrations ("high," "medium," and "low") of 2,4-dichlorophenol (Exper. U-7, U-8, and U-9) and butanethiol (Exper. U-1, U-2, and U-3.)

Table 3. Experimental conditions and results of tests in which trout were exposed to 1, 10, and 100 ppb of 2,4-dichlorophenol for 24 hours, removed, and placed in fresh water for various periods of time.

Concentration (ppb)		Clearing time (hrs.)	Mean off-flavor index[1] (0-6)	LSD[2] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Dissolved oxygen (mg/l)
C-1	2,4-Dichlorophenol		February 5, 1970					
	100	0.25	3.90	1.18	0.44	170	1	11.7
	100	1.27	3.60		0.42	126	1	11.7
	100	6.5	2.40	*	0.56	154	1	11.7
	100	33.5	1.10	*	0.33	115	1	9.9
	100	168	1.70	*	0.34	148	1	10.8
C-2	2,4-Dichlorophenol		February 5, 1970					
	10	0.25	2.40	n.s.	0.43	165	1	10.6
	10	1.27	2.15		0.37	176	1	10.6
	10	6.5	1.20		0.27	168	1	10.6
	10	33.5	1.40		0.36	206	1	8.1
	10	168	1.20		0.25	238	1	9.6
C-3	2,4-Dichlorophenol		February 5, 1970					
	1	0.25	1.80	n.s.	0.40	137	1	11.8
	1	1.27	1.55		0.25	166	1	11.8
	1	6.5	1.65		0.22	131	1	11.8
	1	33.5	1.20		0.23	155	1	11.2
	1	168	1.65		0.36	143	1	12.4

[1] Off-flavor index based on a scale of 0 (no off-flavor) to 6 (very extreme off-flavor). Values based on 10 judgments.

[2] Least significant difference at P=0.05 based on a two-way analysis of variance. An asterisk (*) indicates a statistical significant change in flavor.

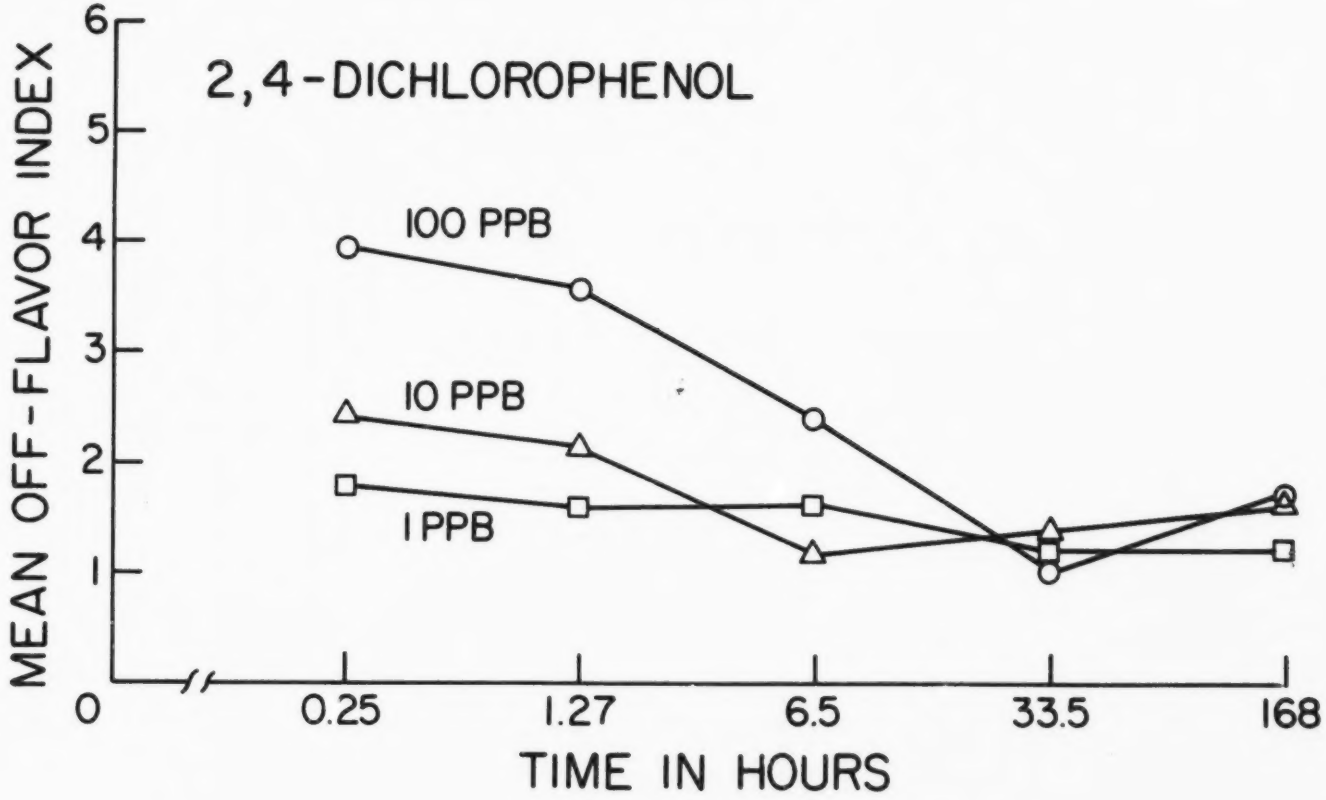

Figure 6. Mean off-flavor indices in relation to the length of time trout were held in fresh water after 24 hour exposure to 2,4-dichlorophenol concentrations of 100, 10, and 1 ppb (Exper. C-1, C-2, and C-3).

The results of the experiment on clearing rate revealed that tainting of the flesh of trout caused by exposure to 2,4-dichlorophenol is eliminated rather rapidly once the fish is placed in fresh water. Trout appear to obtain off-flavor from exposure to 2,4-dichlorophenol much more rapidly than they lose it.

The rates at which taining substances are cleansed from the flesh of fish may vary substantially. Korschgen, Baldwin, and Robinson (1970) reported that the flavor of contaminated (tainted) carp failed to improve after 18 days retention in a clean-water pond. They also reported a study by Leslie E. Whitesel, in which she reported that catfish transferred from the Ohio River to clean water lost about one half of their off-flavor in 7 days, and nearly all in 21 days. Shumway (1966) reported results similar to those found in the study reported here in experiments with phenolic wastes discharged from a plant producing pesticides. The major flavor impairing substance in the waste was 2,4-dichlorophenol. Much remains to be learned about the rate fish pick up and lose tainting substances.

Weight of fish per chamber: A series of experiments were conducted to determine the weight of fish that could be held in the exposure chambers without influencing the degree of flavor impairment. Experiments were conducted with p-chlorophenol, 2,4-dichlorophenol, and pyridine. In each series of tests, trout of about the same size were selected from the stock tanks and placed in the exposure chambers. The desired weight of fish per chamber was attained by varying the number of fish rather than the size of fish. In tests with p-chlorophenol (1,000, 100, and 10 ppb) and 2,4-dichlorophenol (100 and 10 ppb) the weight of fish ranged from about 135 g to somewhat over 1,000 g per chamber; the range of weights used in the tests with pyridine was about 300 to 1,900 g, although this varied from test to test. Each chamber held 70 liters of test solution and was resupplied at a rate of 250 ml/minute. The trout were exposed for 48 or 96 hours at 15°C. The results of the experiments described above are presented in Table 4.

The off-flavor indices for trout exposed to the various concentrations at the three organic compounds tested varied with concentration as expected, but they did not vary with increasing weight of fish. The results suggest that as much as 1,000 to 2,000 grams of fish could be exposed in chambers without concern for the influences such relatively large weights of fish might have on the degree of flavor impairment.

Dissolved oxygen concentration: A number of experiments were conducted to determine the influence of moderate reduction of dissolved oxygen concentration on the flavor-imparting capacity of 2,4-dichlorophenol and pyridine. Trout were exposed to 100, 10, and 1 ppb of 2,4-dichlorophenol and 100, 10, and 1 ppm of pyridine at three dissolved oxygen concentrations ranging from near air-saturation to as low as 3.0 mg/liter in some experiments (Table 5). The trout were exposed for 48 hours at 15°C.

Table 4. The experimental conditions and results of test in which different weights of rainbow trout were exposed to selected concentrations of p-chlorophenol, 2,4-dichlorophenol, and pyridine at 15°C.

Exper. no.	Concentration ppb	Concentration ppm	Mean off-flavor index[1/] (0-6)	Fish per chamber grams	Fish per chamber number	LSD[2/] .05	Standard error of the mean	Dissolved oxygen (mg/l)	pH
W-1	p-Chlorophenol	May 18, 1971	48-Hr. Exposure						
	10		1.20	138	1	n.s.	0.52	8.8	7.7
	10		1.20	258	2		0.52	8.6	7.9
	10		1.30	602	4		0.31	8.2	7.4
	10		0.80	1037	8		0.39	6.5	7.7
W-2	p-Chlorophenol	May 18, 1971	48-Hr. Exposure						
	100		1.55	132	1	n.s.	0.36	9.8	8.0
	100		1.55	233	2		0.46	9.4	7.8
	100		1.85	588	4		0.44	7.5	7.8
	100		2.45	1094	8		0.49	6.7	7.8
W-3	p-Chlorophenol	May 18, 1971	48-Hr. Exposure						
	1,000		2.40	133	1	0.90	0.40	9.4	8.0
	1,000		1.95	239	2		0.57	9.5	7.7
	1,000		3.50	513	4	*	0.51	7.8	7.5
	1,000		3.10	1185	8		0.59	3.5	7.4
W-4	2,4-Dichlorophenol	July 9, 1970	96-Hr. Exposure						
	10		1.85	137	1	n.s.	0.42	9.7	-
	10		0.75	462	3		0.28	8.3	-
	10		0.90	844	5		0.28	14.6	-
	10		0.70	1418	8		0.20	13.7	-

Table 4. Continued

Exper. no.	Concentration ppb	Concentration ppm	Mean off-flavor index (0-6) [1]	Fish per chamber grams	Fish per chamber number	LSD.05 [2]	Standard error of the mean	Dissolved oxygen (mg/l)	pH
W-5	2,4-Dichlorophenol	July 9, 1970	96-Hr. Exposure						
	100		2.10	133	1	n.s.	0.34	9.8	-
	100		3.15	402	3		0.33	3.9	-
	100		1.65	708	5		0.40	10.4	-
	100		2.40	1279	8		0.36	11.9	-
W-6	Pyridine	June 3, 1970	48-Hr. Exposure						
		1	2.00	315	1	n.s.	0.33	9.2	-
		1	1.80	965	3		0.40	6.4	-
		1	1.90	1876	5		0.30	4.0	-
W-7	Pyridine	June 3, 1970	48-Hr. Exposure						
		10	2.30	397	1	n.s.	0.34	7.3	-
		10	1.85	1187	3		0.36	5.3	-
		10	1.60	1688	5		0.30	4.3	-
W-8	Pyridine	June 3, 1970	48-Hr. Exposure						
		100	2.95	309	1	n.s.	0.35	9.7	-
		100	3.15	976	3		0.32	5.8	-
		100	3.20	1986	5		0.55	3.0	-

[1] Off-flavor index based on a scale of 0 (no off-flavor) to 6 (very extreme off-flavor). Values are based on 10 judgments.

[2] The least significant difference at P=0.05 based on a two-way analysis of variance. An asterisk (*) indicates a statistically significant difference in flavor.

Table 5. The results and experimental conditions of tests with rainbow trout held at different dissolved oxygen concentrations and exposed for 48 hours concentration of 2,4-dichlorophenol and pyridine. Water temperatures and pH values were near 15°C and 7.8-8.0, respectively.

Exper. no.	Concentration ppb	Concentration ppm	Mean off-flavor index[1] (0-6)	Mean dissolved oxygen (mg/l)	LSD[2] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	pH
D-1	2,4-Dichlorophenol	August 12, 1970	10 Judgments						
	10		1.75	5.1	*	0.30	481	1	-
	10		0.80	6.4		0.34	350	1	-
	10		0.80	9.9	0.59	0.17	328	1	-
D-2	2,4-Dichlorophenol	August 12, 1970	10 Judgments						
	100		3.85	5.8	*	0.32	448	1	-
	100		3.75	6.0	*	0.33	361	1	-
	100		2.60	10.4	0.62	0.27	302	1	-
D-3	2,4-Dichlorophenol	March 10, 1971	10 Judgments						
	1		0.55	4.12		0.25	374	2	-
	1		1.00	6.50		0.30	311	2	-
	1		0.60	10.35	n.s.	0.16	316	2	-
D-4	2,4-Dichlorophenol	March 10, 1971	10 Judgments						
	10		1.85	3.92		0.39	351	2	-
	10		2.35	6.90		0.42	355	2	-
	10		1.35	10.40	n.s.	0.25	381	2	-
D-5	2,4-Dichlorophenol	March 10, 1971	10 Judgments						
	100		3.60	3.88		0.54	341	2	-
	100		3.80	6.20		0.33	398	2	-
	100		3.55	10.62	n.s.	0.50	379	2	-

Table 5. Continued

Exper. no.	Concentration ppb	Concentration ppm	Mean off-flavor index[1/] (0-6)	Mean dissolved oxygen (mg/1)	LSD[2/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	
D-6 Pyridine	August 15, 1970		10 Judgments						
		10	0.50	5.2		0.26	405	1	-
		10	1.20	6.5		0.52	348	1	-
		10	0.70	9.7	n.s.	0.21	453	1	-
D-7 Pyridine	August 15, 1970		10 Judgments						
		100	3.70	5.1		0.44	359	1	-
		100	2.50	6.4		0.43	339	1	-
		100	3.40	10.7	n.s.	0.45	379	1	-
D-8 Pyridine	March 18, 1971		Trout 11 Judgments						
		1	0.68	3.05		0.18	336	2	-
		1	0.59	6.00		0.20	381	2	-
		1	1.09	9.82	n.s.	0.52	493	2	-
D-9 Pyridine	March 18, 1971		Trout 11 Judgments						
		10	0.55	3.17		0.22	352	2	-
		10	1.32	6.05		0.45	368	2	-
		10	0.77	10.53	n.s.	0.18	347	2	-

Table 5. Continued

Exper. no.	Concentration ppb	Concentration ppm	Mean off-flavor index[1/] (0-6)	Mean dissolved oxygen (mg/1)	LSD[2/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	pH
D-10	Pyridine	March 18, 1971	Trout	11 Judgments					
		100	4.18	3.06		0.44	355	2	-
		100	3.95	5.20		0.56	370	2	-
		100	2.82	10.7	n.s.	0.58	308	2	-

1/ Off-flavor index based on a 0 to 6 scale, with 0 representing no off-flavor and 6 very extreme off-flavor.

2/ Least significant difference (P=0.05) based on a two-way analysis of variance. An asterisk (*) indicates a statistical difference in the flavor.

In general, the results of the experiments with pyridine indicate that moderately to substantially reduced dissolved oxygen concentrations will not influence the degree of flavor impairment of the flesh of trout held at concentrations of 100, 10, and 1 ppm. In some experiments with 2,4-dichlorophenol, however, fairly good correlation was noted between reduction of dissolved oxygen concentration and the degree of flavor impairment. In other experiments with 2,4-dichlorophenol, however, exposure to moderately reduced dissolved oxygen concentrations had little or no influence on the degree of flavor impairment.

In light of the conclusive results obtained in the experiments described above with reduced dissolved oxygen concentrations, a firm conclusion could not be reached concerning the influence of even moderate reductions of dissolved oxygen on the degree of flavor impairment of the flesh of trout. Since the dissolved oxygen concentration of the water in the exposure chamber may influence the results of flavor experiments, and since trout seem to withstand the stress of handling better at relatively high dissolved oxygen concentrations, concentration in the exposure chambers in subsequent testings was held at or near air-saturation.

Influence of pH: The degree of ionization of an electrolyte is affected by the pH of the media into which it is introduced. If the dissociation product of a particular contaminant has a greater or lesser capacity to impart off-flavor than the undissociated chemical, the pH of the solution will be an important factor in determining the flavor-imparting capacity of the chemical in question.

A series of experiments were conducted on the influence of pH on the degree of flavor impairment of trout held at 100 and 10 ppb of 2,4-dichlorophenol and 100 and 10 ppm of pyridine at "high" (8.8 to 9.3), "normal" (7.6 to 8.0) and "low" (4.9 to 6.5) pH levels. The experiments ranged in length from 24 to 96 hours. Sodium hydroxide and sulfuric acid were used to adjust the pH of the incoming water. The experimental condition and results of the experiments are presented in Table 6.

In the experiments with pyridine, a weak base, an increase of pH from "normal" to about 9.3 resulted in little or no change in the degree of flavor impairment at either test concentration. A decrease in pH from "normal," however, caused a slight decrease in off-flavor at the 100 ppm level, but the same decrease in pH caused a slight increase in off-flavor at 10 ppm (See Figure 7). The divergent results, neither of which proved significant at the 5 percent level (Table 6), suggest that pH has no effect on the flavor-imparting capacity of pyridine.

The off-flavor index of trout exposed to 100 ppb of 2,4-dichlorophenol, a weak acid, decreased with a change of pH from the "low" level to the "high" level. The length of exposure time (24, 48, and 96 hours) did not appear to alter the degree of flavor impairment. Changes in pH did not appear to influence the degree of flavor-impairment at the lower concentration of 2,4-dichlorophenol (10 ppb). The results of two tests

Table 6. Conditions and results of tests in which rainbow trout were exposed to a concentration of 2,4-dichlorophenol or pyridine maintained at a high, intermediate or low pH value and at a temperature of 15°C.

Exper. no.	Concentration ppb	ppm	Mean off-flavor index[1] (0-6)	Mean pH	LSD[2] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Dissolved oxygen
P-1	2,4-Dichlorophenol	July 15, 1970	24-Hr. Exposure						
	10		0.40	8.88		0.12	268	1	10.0
	10		1.45	7.79	n.s.	0.39	331	1	10.4
	10		1.75	6.45		0.32	293	1	11.0
P-2	2,4-Dichlorophenol	July 15, 1970	24-Hr. Exposure						
	100		1.05	8.87	*	0.25	288	1	10.4
	100		2.95	7.66	0.74	0.44	305	1	9.4
	100		3.75	6.26	*	0.40	303	1	10.2
P-3	2,4-Dichlorophenol	July 15, 1970	48-Hr. Exposure						
	10		1.00	8.90		0.25	342	1	9.8
	10		0.50	8.01	n.s.	0.24	330	1	10.2
	10		2.05	6.39		0.46	305	1	10.6
P-4	2,4-Dichlorophenol	July 15, 1970	48-Hr. Exposure						
	100		1.40	8.88	*	0.26	261	1	10.0
	100		3.40	7.83	0.92	0.54	324	1	9.3
	100		3.95	6.28	*	0.36	376	1	9.5
P-5	2,4-Dichlorophenol	July 20, 1970	96-Hr. Exposure						
	10		0.75	8.92	n.s.	0.30	286	1	9.8
	10		1.00	7.93		0.22	408	1	10.2
	10		1.05	6.21		0.27	267	1	10.4

Table 6. Continued

Exper. no.	Concentration ppb	Concentration ppm	Mean off-flavor index[1] (0-6)	Mean pH	LSD[2] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Dissolved oxygen
P-6	2,4-Dichlorophenol	July 20, 1970	96-Hr. Exposure						
	100		2.15	8.88	*	0.61	414	1	9.9
	100		3.50	7.97	0.87	0.48	274	1	9.7
	100		2.60	6.11	*	0.45	387	1	10.5
P-7	Pyridine	August 3, 1970	48-Hr. Exposure						
		10	0.75	9.3	n.s.	0.38	309	1	10.1
		10	0.75	7.9		0.29	356	1	10.2
		10	1.55	4.9		0.58	449	1	10.3
P-8	Pyridine	August 3, 1970	48-Hr. Exposure						
		100	3.05	9.3	n.s.	0.65	375	1	10.4
		100	2.95	7.8		0.57	553	1	9.3
		100	2.25	5.7		0.47	274	1	10.5

[1] Off-flavor index based on a scale of 0 (no off-flavor) to 6 (very extreme off-flavor). In each test the number of judgments was 10.

[2] The least significant difference at P=0.05 based on a two-way analysis of variance. An asterisk (*) indicates a statistically significant change in flavor.

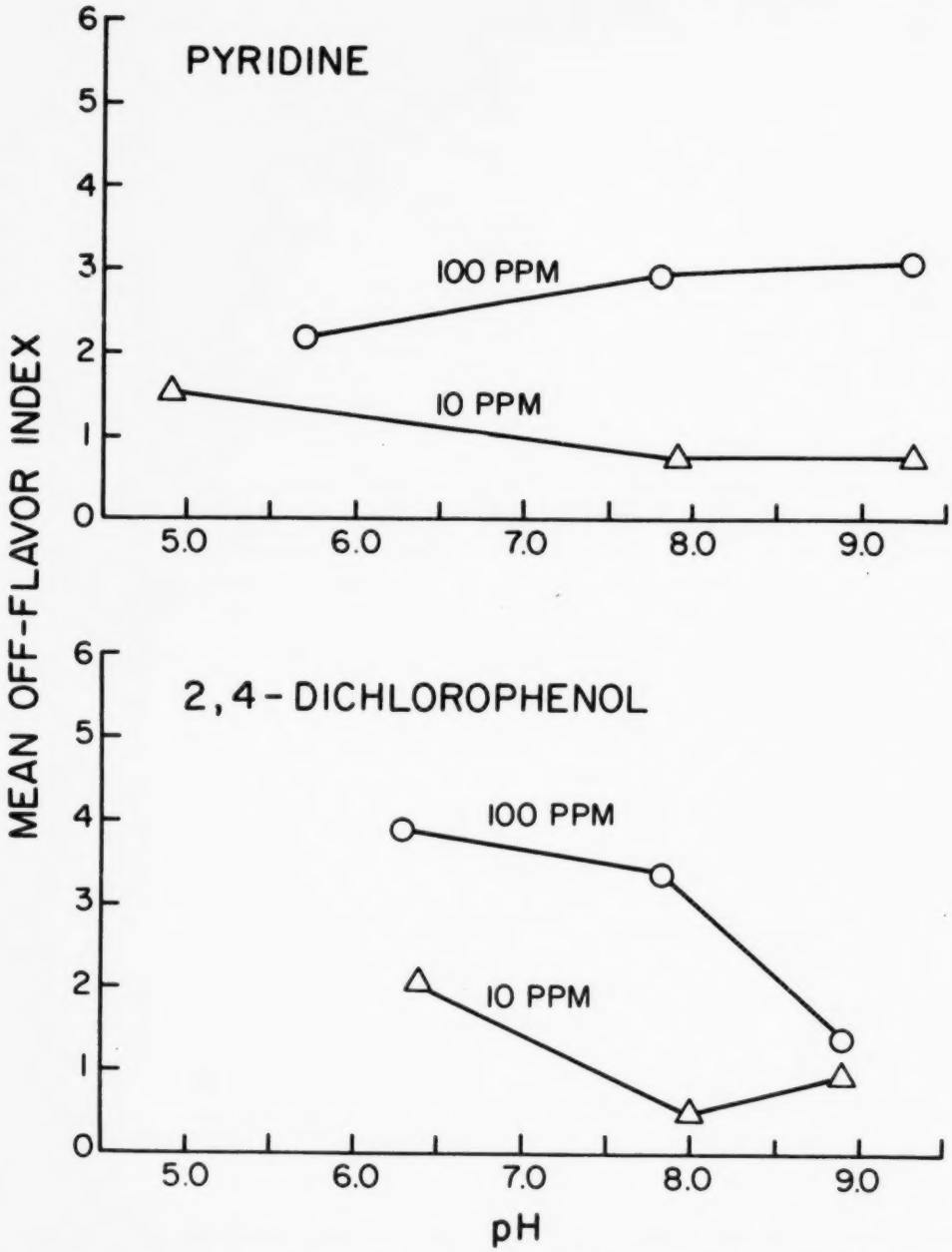

Figure 7. The relation between mean off-flavor indices and the pH at which trout were held and exposed to 100 and 10 ppm of pyridine (Exper. P-7 and P-8) and 100 and 10 ppb of 2,4-dichlorophenol (Exper. P-3 and P-4).

with 2,4-dichlorophenol are presented graphically in Figure 7.

2,4-dichlorophenol, which is quite soluble in fat, partially dissociates to 2,4-dichlorophenate, a compound readily soluble in water, but nearly insoluble in fat. As the dissociation equilibrium shifts with increasing pH, more 2,4-dichlorophenate is formed and less 2,4-dichlorophenol remains in the solution for the fish to remove and concentrate in their tissue.

pH is known to influence the toxicity of a number of compounds. The toxicity of other material is unaltered by change in the pH. The same appears to be true with the effect of pH on the flavor-imparting capacity of organic compounds. In laboratory experiments designed to determine threshold concentrations for compounds, the pH probably should be maintained between 7.0 and 8.0. Where the impact of a contaminant on a particular body of water is under study, the pH of the receiving water should be used.

Influence of organic compounds on flavor: Once a standard procedure was selected for use, examination of the flavor imparting capacity of a number of organic compounds and effluents could commence. Rainbow trout and in a few cases, largemouth bass and bluegill, were exposed to a range of concentration of a compound and a control for 48 hours. The pH was maintained between 7.0 and 8.0, and the water temperature was held at 15° C for trout and 20° C for bluegill and bass. Since most of the contaminants tested had not been previously examined for their tainting characteristic, the ranges of concentrations used were often selected quite arbitrarily. In some cases, available toxicity data allowed the setting of the upper limit of the range tested.

Presented in Table 7 are the experimental conditions and results of the tests conducted with organic compounds. Also presented in Table 7 are the statistical evaluations of the results of each test and other pertinent information. As may be seen in Table 7, experiments with many organic compounds had to be repeated in order to obtain meaningful results. Other experiments were duplicated using either bass or bluegill as the test fish.

The number of judges used to evaluate the samples of fish proved to be an important factor in obtaining meaningful results. Initially, the flavor panels were comprised of 8 to 10 judges. This was later changed to a minimum of fifteen judges. Because of the subjective nature of the tests, the larger panels proved far more satisfactory than did the small panels.

Paper processing effluents: Biologically stabilized effluents were obtained from paper mills located near Halsey, Albany, and Lebanon, Oregon, and evaluated for their flavor-imparting capacity. The experimental conditions and results of the experiment are presented in Table 8.

Table 7 . Experimental conditions and results of tests in which fish were exposed to various concentrations of chemicals for 48 hours.

Exper. No.		Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index[1/2] (0-6)	LSD[3/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4/] (C)	Dissolved oxygen (mg/1)	pH
T-1	Acetone	June 29, 1971	Trout	11 Judgments							
		0		0.82	n.s.	0.22	335	2	-	-	7.6
		1		0.82		0.22	294	2	-	-	7.7
		10		1.91		0.50	284	2	-	-	7.6
		100		0.77		0.24	340	2	-	-	7.7
		1,000		0.68		0.23	337	2	-	-	7.7
		10,000		+			-	-	-	-	-
T-2	Acrylonitrile	March 3, 1971	Trout	10 Judgments							
		0		1.90	n.s.	0.49	240	2	-	11.0	7.9
		0.1		1.00		0.31	294	2	-	10.0	7.9
		1.0		0.90		0.31	329	2	-	10.2	7.9
		10		2.00		0.50	317	2	-	10.0	7.9
		100		+		-	-	2	-	10.2	7.9
T-3	Acrylonitrile	April 21, 1971	Trout	11 Judgments							
		0		0.41	0.70	0.15	254	2	-	9.0	7.9
		0.32		2.32	*	0.50	332	2	-	9.9	7.9
		0.56		0.77		0.26	346	2	-	10.0	7.8
		3.2		0.55		0.21	270	2	-	10.6	7.8
		5.6		0.95		0.18	313	2	-	10.0	7.9
		32.0		1.41	*	0.25	215	2	-	10.1	8.0
		56.0		+		-	-	-	-	-	-

Table 7 Continued

Exper. No.	Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index 1/ (0-6)	LSD 3/ .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp. 4/ (C)	Dissolved oxygen (mg/1)	pH
T-4 Amyl Acetate	May 26, 1971	Trout	11 Judgments							
	0		0.36	n.s.	0.14	393	3	-	9.4	7.6
	0		1.04		0.24	380	3	-	9.6	7.8
	0.1		0.41		0.16	289	2	-	9.2	8.1
	1		0.59		0.30	254	2	-	9.5	7.9
	10		0.91		0.31	246	2	-	8.6	7.9
	100		0.86		0.30	237	2	-	9.2	7.8
	1,000		0.95		0.18	266	2	-	8.7	7.8
	10,000		1.36		0.34	264	2	-	8.7	7.7
T-5 Aniline	December 30, 1970	Trout	8 Judgments							
	0		0.69	n.s.	0.27	534	2	-	8.9	-
	0.1		0.88		0.25	433	2	-	9.1	-
	1		0.69		0.23	404	2	-	10.0	-
	10		1.63		0.25	454	2	-	9.8	-
	100		+		-	-	2	-	10.5	-
T-6 Benzene	February, 7, 1972	Trout	16 Judgments							
	0		1.06	n.s.	0.27	579	5	-	10.4	7.7
	0.01		0.62		0.13	620	5	-	9.0	7.6
	0.056		1.53		0.29	697	5	-	9.4	7.9
	0.10		0.75		0.17	543	5	-	8.8	7.8
	.56		0.56		0.16	623	5	-	6.0	7.9
	1.00		1.47		0.30	690	5	-	7.6	7.5
	5.60		0.78		0.20	640	5	-	9.8	7.6
T-7 n-Butanol	September 13, 1971	Bluegill	12 Judgments							
	0		0.17	n.s.	0.09	745	9	-	8.3	7.3
	0.1		0.54		0.23	648	8	-	8.5	7.4
	1		0.42		0.17	562	6	-	7.7	7.1
	10		0.25		0.12	753	8	-	10.2	7.2
	100		0.54		0.18	808	9	-	11.0	7.2

Table 7 Continued

Exper. no.	Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index[1/2]	LSD[3] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4] (C)	Dissolved oxygen (mg/l)	pH
T-8 Cresol	September 20, 1971	Trout	15 Judgments							
	0		0.70	0.71	0.21	759	5	-	9.7	7.2
	0.005		0.80		0.27	737	5	-	9.0	7.1
	0.05		0.83		0.21	817	5	-	9.4	7.2
	0.5		2.33	*	0.33	777	5	-	10.8	7.1
	5.0		4.33	*	0.31	760	4	-	10.0	7.0
T-9 Cresol	September 28, 1971	Trout	15 Judgments							
	0		0.47	0.73	0.14	721	5	-	5.9	7.2
	0.005		0.90		0.29	834	5	-	5.6	7.2
	0.05		1.30	*	0.24	819	5	-	6.6	7.4
	0.5		2.33	*	0.37	797	5	-	6.6	7.3
	5.0		4.90	*	0.26	699	4	-	6.4	7.3
T-10 m-Cresol	September 8, 1971	Trout	20 Judgments							
	0		0.82	0.63	0.18	673	5	-	8.3	7.4
	0.005		0.75		0.22	768	5	-	7.1	7.3
	0.05		0.78		0.13	652	5	-	8.7	7.4
	0.5		2.12	*	0.33	870	5	-	8.7	7.1
	5.0		3.77	*	0.31	946	5	-	7.0	7.2
T-11 m-Cresol	September 8, 1971	Trout	15 Judgments							
	0		0.70	0.77	0.22	756	5	-	7.4	7.3
	0.005		0.70		0 14	794	5	-	7.8	7.3
	0.05		0.93		0.22	799	5	-	7.9	7.2
	0.5		1.90	*	0.41	896	5	-	8.1	7.2
	5.0		3.93	*	0.32	811	5	-	7.7	7.0

Table 7 Continued

Exper. no.	Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index[1/2/] (0-6)	LSD[3/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4/] (C)	Dissolved oxygen (mg/1)	pH
T-12	p-Cresol	September 20, 1971	Trout 16 Judgments							
		0	0.56	0.82	0.13	661	4	-	9.9	7.5
		0.005	0.87		0.21	1067	5	-	9.3	7.3
		0.05	0.84		0.39	898	5	-	9.6	7.4
		0.50	2.91	*	0.46	727	4	-	9.4	7.2
		5.0	+		-	-	-	-	-	-
T-13	p-Cresol	September 20, 1971	Trout 15 Judgments							
		0	0.47	0.81	0.12	854	5	-	9.1	7.2
		0.005	2.17	*	0.30	788	5	-	8.9	7.1
		0.05	0.90		0.37	745	5	-	10.3	7.3
		0.50	2.20	*	0.40	1082	5	-	9.5	7.2
		5.0	+		-	-	-	-	-	-
T-14	Dimethylamine	August 4, 1971	Trout 12 Judgments							
		0	0.08	0.59	0.06	662	5	-	6.9	7.6
		0.56	0.71	*	0.25	675	5	-	7.7	7.4
		5.6	0.66		0.26	666	5	-	8.9	7.8
		56	1.66	*	0.30	645	5	-	8.0	9.2
T-15	Ethanethiol	October 5, 1971	Trout 15 Judgments							
	0		1.03	0.75	0.29	914	5	-	6.1	7.2
	1		0.70		0.17	785	5	-	7.9	7.5
	10		1.63		0.38	980	5	-	7.1	7.4
	100		0.73		0.22	899	5	-	5.7	7.3
	1,000		2.43	*	0.43	902	5	-	5.9	6.9
T-16	Ethanethiol	October 5, 1971	Trout 15 Judgments							
		0	3.00	0.79	0.30	887	5	-	4.9	7.4
		1	0.97	*	0.29	894	5	-	5.1	7.3
		10	0.90	*	0.26	676	5	-	6.7	7.3
		100	1.37	*	0.41	821	5	-	5.5	7.1
		1,000	2.53		0.28	915	5	-	6.7	7.1

Table 7. Continued

Exper. no.	Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index[1/2/] (0-6)	LSD[3/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4/] (C)	Dissolved oxygen (mg/l)	pH
T-17	Ethylacrylate	August 24, 1971	Trout	15 Judgments						
	0		0.93	n.s.	0.24	732	5	15.8	10.2	7.5
	1		1.13		0.31	692	5	15.8	11.0	7.6
	10		1.20		0.45	644	5	15.9	12.5	7.6
	100		0.87		0.19	827	5	15.8	11.4	7.4
T-18	Ethylacrylate	August 24, 1971	Trout	21 Judgments						
	0		0.71	0.53	0.17	741	5	-	15.0	7.6
	1		0.83		0.16	703	5	-	11.8	7.6
	10		0.69		0.14	724	5	-	16.6	7.5
	100		1.81	*	0.27	810	5	-	12.3	7.7
T-19	Ethylacrylate	September 28, 1971	Trout	18 Judgments						
		0	0.81	0.68	0.22	676	5	-	7.0	7.3
		0.01	0.86		0.18	671	5	-	6.4	7.4
		0.1	0.83		0.22	742	4	-	7.0	7.6
		1	1.17		0.24	797	5	-	5.6	7.5
		10	3.25	*	0.38	359	2	-	6.8	7.2
T-20	Ethylacrylate	September 28, 1971	Trout	17 Judgments						
		0	1.24	0.65	0.31	676	5	-	7.0	7.3
		0.01	1.06		0.25	671	4	-	6.4	7.4
		0.10	1.00		0.24	742	4	-	7.0	7.6
		1	1.97	*	0.29	797	5	-	5.6	7.5
		10	3.15	*	0.37	359	2	-	6.8	7.2
T-21	Formaldehyde	April 28, 1971	Trout	10 Judgments						
		0	0.75	n.s.	0.33	438	3	-	7.3	7.8
		0.10	2.10		0.48	315	2	-	10.0	7.9
		0.56	1.15		0.34	477	3	-	10.4	8.1
		1.0	1.05		0.24	287	2	-	10.0	8.0

Table 7. Continued

Exper. no.	Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index 1/2/ (0-6)	LSD .05 3/	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp. 4/ (C)	Dissolved oxygen (mg/1)	pH
T-22	Formaldehyde	April 28, 1971	Trout	10 Judgments						
	0		0.55	0.69	0.24	254	2	-	9.2	7.7
	5.6		0.80		0.17	221	2	-	9.8	7.8
	10		0.55		0.23	352	2	-	9.7	7.8
	56		0.50		0.21	395	3	-	9.4	8.0
	100		2.05	*	0.40	299	3	-	9.9	8.1
T-23	Formaldehyde	May 12, 1971	Trout	10 Judgments						
	0		0.50	n.s.	0.26	528	2	-	9.2	7.8
	32		0.75		0.25	299	1	-	10.3	7.9
	100		1.00		0.31	314	1	-	10.2	8.0
	320		+		-	-	-	-	-	-
T-24	β, β'-dichlorodiethyl ether		March 22, 1971	Trout	10 Judgments					
	0		0.40	0.96	0.15	547	3	-	8.6	7.9
	.09		0.45		0.17	351	2	-	9.3	7.8
	0.9		1.40	*	0.41	396	2	-	8.1	8.0
	9		2.90	*	0.50	362	2	-	9.3	7.8
	91		+		-	-	2	-	9.8	8.0
T-25	Methylamine	January 5, 1971	Trout	8 Judgments						
	0		1.06	n.s.	0.29	558	2	-	8.2	7.9
	0.001		1.00		0.34	355	2	-	9.4	7.9
	0.01		1.50		0.37	390	2	-	10.1	7.9
	0.10		1.06		0.49	505	2	-	10.2	7.9
	1		1.38		0.34	556	2	-	10.0	8.0
	10		1.69		0.35	473	2	-	10.6	8.4

Table 7. Continued

Exper. no.	Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index[1/2/] (0-6)	LSD[3/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4/] (C)	Dissolved oxygen (mg/1)	pH
T-26 Methylamine	March 3, 1971 Trout			11 Judgments						
		0	0.73	n.s.	0.24	321	2	-	9.0	7.8
		0.056	1.45		0.46	326	2	-	9.2	7.8
		0.56	1.50		0.42	348	2	-	10.0	7.8
		5.60	1.59		0.31	315	2	-	10.0	8.2
		56.0	+		-	-	-	-	10.0	9.5
T-27	2-Ethyl-1-hexanol	February 8, 1972	Trout	12 Judgments						
		0	1.21	n.s.	0.43	713	5	-	10.2	7.8
		0.0056	0.83		0.24	736	5	-	10.4	7.8
		0.056	0.83		0.29	781	5	-	10.4	7.7
		0.56	1.17		0.29	870	5	-	9.6	7.9
		5.6	1.21		0.27	668	5	-	9.6	7.4
T-28	2-Napthol	February 21, 1972	Trout	15 Judgments						
		0	1.20	0.68	0.40	644	5	-	11.6	7.8
		0.01	0.63		0.21	841	5	-	11.4	7.8
		0.032	1.33		0.21	620	5	-	10.6	7.9
		0.10	0.87		0.25	693	5	-	10.4	8.0
		0.32	1.53		0.45	961	5	-	10.2	7.7
		1.0	2.40	*	0.52	584	5	-	11.4	7.7
		3.2	+		-	-	-	-	-	-
T-29	Sodium pentachlorophenate	November 22, 1971	Trout	15 Judgments						
	0		1.03	n.s.	0.22	601	6	15.0	7.8	7.5
	0.2		0.97		0.19	537	5	15.6	7.0	7.4
	2		0.96		0.19	525	5	15.6	6.6	7.5
	20		0.83		0.26	600	5	15.6	5.4	7.3
	200		+		-	-	-	-	5.2	7.2

Table 7. Continued

Exper. no.	Exposure concentration ppb	ppm	Mean off-flavor index[1/2] (0-6)	LSD[3] .05	Standard error of the mean	Fish per chamber grams	number	Mean temp.[4] (C)	Dissolved oxygen (mg/1)	pH
T-30	Phenol	March 23, 1971	Trout	10 Judgments						
	0		0.95	n.s.	0.22	331	2	-	8.2	7.9
	1.0		1.55		0.66	410	2	-	8.9	7.7
	10		1.30		0.41	369	2	-	9.9	7.7
	100		0.90		0.29	366	2	-	9.5	8.0
T-31	Phenol	June 23, 1971	Trout	12 Judgments						
		0	0.58	n.s.	0.32	454	2	-	9.4	7.9
		0.10	0.46		0.18	142	1	-	9.8	8.1
		0.18	1.33		0.49	324	2	-	9.5	7.8
		0.56	1.08		0.31	273	2	-	9.3	8.0
		1.00	1.29		0.27	289	2	-	9.7	7.8
		1.80	0.75		0.20	335	2	-	9.1	7.9
		5.60	1.08		0.45	302	2	-	9.4	8.0
T-32	Phenol	April 14, 1971	Trout	10 Judgments						
		0	0.95	n.s.	0.19	400	3	-	9.5	7.9
		0.01	0.35		0.13	238	2	-	10.4	7.5
		0.1	0.55		0.20	285	2	-	10.2	8.1
		0.32	0.80		0.32	322	2	-	11.2	8.0
		1.0	0.50		0.26	288	2	-	10.3	7.9
		3.2	1.05		0.25	315	2	-	10.2	7.7
		10.0	+		-	-	-	-	-	-
		32	+		-	-	-	-	-	-
T-33	2,3-Dichlorophenol	March 1, 1972	Trout	15 Judgments						
		0	0.63	0.64	0.25	792	5	-	11.4	7.7
		0.01	0.70		0.18	654	5	-	10.2	7.6
		0.032	0.77		0.21	786	5	-	9.2	7.7
		0.10	1.43	*	0.27	831	5	-	10.0	7.4
		0.32	3.20	*	0.41	631	5	-	10.0	7.5
		1.0	5.13	*	0.24	714	5	-	10.8	7.5

Table 7. Continued

Exper. no.	Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index[1/2/] (0-6)	LSD[3/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4/] (C)	Dissolved oxygen (mg/1)	pH
T-34	2,4-Dichlorophenol		August 26, 1970 Bass 10 Judgments							
	0		0.40	0.88	0.19	802	2	19.8	8.0	-
	0.01		1.55	*	0.29	546	2	19.8	8.8	-
	0.1		0.90		0.39	509	2	19.8	9.0	-
	1		2.10	*	0.35	477	2	19.7	8.8	-
	10		1.75	*	0.36	472	2	19.3	9.0	-
	100		3.75	*	0.32	520	2	18.7	8.0	-
T-35	2,4-Dichlorophenol		July 21, 1971 Bluegill 10 Judgments							
	0		0.55	0.71	0.24	446	6	19.8	8.6	7.8
	0.10		0.50		0.21	478	6	19.9	8.5	7.8
	1		0.55		0.22	446	6	19.9	8.1	7.8
	10		1.00		0.34	401	6	19.8	8.5	7.9
	100		3.50	*	0.38	419	6	19.9	8.4	7.9
T-36	2,5-Dichlorophenol		February 28, 1972 Trout 15 Judgments							
	0		0.93	0.76	0.29	714	6	-	10.0	7.5
	1		0.90		0.26	663	5	-	10.6	7.7
	10		1.13		0.24	658	5	-	10.4	7.6
	100		2.80	*	0.39	836	5	-	10.0	7.6
	1,000		4.83	*	0.29	768	5	-	9.6	7.4
T-37	2,6-Dichlorophenol		February 15, 1972 Trout 15 Judgments							
	0		0.73	0.74	0.21	670	5	-	9.2	7.6
	1		0.47		0.12	651	5	-	8.4	7.7
	10		0.80		0.19	544	5	-	9.0	7.8
	100		2.03	*	0.33	608	5	-	9.4	7.6
	1,000		4.0	*	0.44	602	5	-	5.0	7.5

Table 7. Continued

Exper. no.	Exposure concentration ppb	ppm	Mean off-flavor index[1/2] (0-6)	LSD[3] .05	Standard error of the mean	Fish per chamber grams	number	Mean temp.[4] (C)	Dissolved oxygen (mg/l)	pH
T-38	m-Chlorophenol	April 21, 1971		Trout	10 Judgments					
	0		1.15	n.s.	0.45	217	2	-	10.2	8.0
	1		1.50		0.35	303	2	-	9.5	8.0
	10		0.90		0.23	262	2	-	10.4	8.0
	100		0.80		0.24	287	2	-	10.1	8.0
	1,000		2.15		0.53	257	2	-	10.2	8.0
	10,000		+		-	-	-	-	-	-
T-39	m-Chlorophenol	August 17, 1971		Bluegill	13 Judgments					
	0		0.50	n.s.	0.23	292	6	19.7	8.8	8.0
	1		0.92		0.31	364	5	19.6	8.7	8.0
	10		0.50		0.15	412	6	19.6	8.6	8.0
	100		0.23		0.11	454	8	19.4	9.2	8.0
	1,000		0.62		0.23	531	7	19.1	10.0	8.0
T-40	o-Chlorophenol	August 11, 1971		Trout	13 Judgments					
	0		2.23	0.78	0.41	811	5	-	8.6	7.7
	1		0.77	*	0.32	716	5	-	8.1	7.8
	10		0.15	*	0.09	714	5	-	8.1	8.0
	100		1.23	*	0.21	754	5	-	9.4	7.9
	1,000		5.08	*	0.33	742	5	-	7.7	7.6
T-41	o-Chlorophenol	August 11, 1971		Trout	15 Judgments					
	0		1.30	0.65	0.38	725	5	-	7.8	7.6
	1		0.37	*	0.14	753	5	-	8.1	7.9
	10		0.57	*	0.17	856	5	-	7.9	7.7
	100		1.77		0.34	751	5	-	8.3	7.7
	1,000		4.56	*	0.28	766	5	-	6.7	7.5

Table 7. Continued

Exper. no.	Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index[1/2/] (0-6)	LSD[3/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4/] (C)	Dissolved oxygen (mg/l)	pH
T-42 p-Chlorophenol	February 10, 1971		Trout		10 Judgments					
	0		0.95	0.69	0.24	614	2	-	8.3	7.8
	0.1		0.70		0.27	559	2	-	7.7	7.9
	1		0.90		0.32	446	2	-	10.6	8.0
	10		0.95		0.27	751	2	-	9.8	7.8
	100		2.15	*	0.27	657	2	-	9.6	7.8
	1,000		4.75	*	0.31	500	2	-	10.0	7.9
T-43 p-Chlorophenol	February 24, 1971		Trout		10 Judgments					
	0		0.70	0.87	0.37	293	2	-	10.4	7.8
	2.1		0.85		0.27	250	2	-	10.7	7.9
	4.5		0.75		0.24	344	2	-	11.5	8.0
	21		1.10		0.29	286	2	-	10.9	7.9
	45		1.85	*	0.43	369	2	-	11.0	7.8
	210		2.30	*	0.42	312	2	-	11.0	7.8
	450		3.60	*	0.36	304	2	-	10.0	7.9
T-44 p-Chlorophenol	July 14, 1971		Bluegill		10 Judgments					
	0		1.0	n.s.	0.41	457	6	19.6	7.9	7.8
	1		0.55		0.22	269	6	19.6	7.8	7.7
	10		1.45		0.38	501	6	19.6	8.1	7.8
	100		1.25		0.43	502	6	19.7	8.0	7.8
	1,000		2.85		0.42	453	6	19.6	7.8	7.7
T-45 o-Phenylphenol	September 8, 1971		Trout		17 Judgments					
		0	0.76	n.s.	0.24	608	5	-	10.3	7.0
		0.001	1.26		0.28	830	5	-	6.6	7.0
		0.01	0.61		0.21	728	5	-	8.3	7.1
		0.1	0.71		0.23	834	5	-	8.0	7.1
		1	1.74		0.32	813	5	-	7.0	7.0

Table 7. Continued

Exper. no.	Exposure concentration ppb	ppm	Mean off-flavor index[1/2/] (0-6)	LSD[3/] .05	Standard error of the mean	Fish per chamber grams	number	Mean temp.[4/] (C)	Dissolved oxygen (mg/1)	pH
T-46 o-Phenylphenol	September 13, 1971		Trout		15 Judgments					
	0		1.20	n.s.	0.37	701	5	-	9.2	7.3
	0.001		0.87		0.25	622	5	-	9.1	7.1
	0.01		0.63		0.18	761	5	-	11.4	7.2
	0.1		1.13		0.15	797	5	-	12.1	7.0
	1		1.83		0.36	807	5	-	8.4	6.8
T-47 2,4,5-Trichlorophenol	June 16, 1971		Trout		10 Judgments					
	0		0.50	n.s.	0.21	480	3	-	-	7.7
	0.1		0.70		0.26	305	2	-	-	7.8
	1		0.70		0.30	291	2	-	-	7.9
	32		0.50		0.25	432	2	-	-	7.8
	100		0.70		0.25	272	2	-	-	7.9
T-48 2,4,5-Trichlorophenol	June 16, 1971		Trout		10 Judgments					
	0		0.55	n.s.	0.16	425	2	-	-	7.7
	0.01		0.75		0.19	363	2	-	-	8.0
	3.20		0.45		0.17	295	2	-	-	7.8
	10		0.75		0.24	323	2	-	-	8.0
	320		1.30		0.27	322	2	-	-	7.7
	1,000		+		-	-	-	-	-	-

Table 7. Continued

Exper. no.	Exposure concentration ppb	Exposure concentration ppm	Mean off-flavor index[1/2/] (0-6)	LSD[3/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4/] (C)	Dissolved oxygen (mg/1)	pH
T-49	2,4,6-Trichlorophenol		May 12, 1971	Trout	10 Judgments					
	0		0.80	0.69	0.31	473	2	-	9.2	7.7
	0.1		0.60		0.31	290	1	-	9.3	7.9
	1		0.50		0.15	273	1	-	9.4	8.0
	10		1.00		0.24	307	1	-	9.8	8.1
	100		1.60	*	0.31	262	1	-	9.8	8.0
	1,000		3.50	*	0.34	284	1	-	9.8	7.9
T-50	Pyridine		August 3, 1971	Bluegill	10 Judgments					
	0		0.90	0.95	0.30	525	6	19.7	8.0	7.8
	1		0.40		0.16	550	6	19.5	8.2	7.9
	10		0.65		0.21	503	6	19.6	8.9	7.9
	100		3.60	*	0.53	746	6	19.6	8.8	7.9
T-51	Pyrocatechol		January 26, 1972	Trout	15 Judgments					
	0		0.80	0.68	0.18	566	6	-	7.4	7.3
	0.32		0.93		0.21	431	5	-	13.4	7.2
	1.0		2.07	*	0.33	628	6	-	8.0	7.3
	3.2		2.13	*	0.32	461	5	-	13.8	7.1
	10		+		-	-	-	-	-	-
	32		+		-	-	-	-	-	-

Table 7. Continued

Exper. no.	Exposure concentration ppb	ppm	Mean off-flavor index[1/] (0-6)	LSD[3/] .05	Standard error of the mean	Fish per chamber grams	number	Mean temp.[4/] (C)	Dissolved oxygen (mg/1)	pH
T-52 Guaiacol	April 25, 1972 Trout 15 Judgments									
	0		0.83	0.78	0.27	650	5	-	10.5	7.8
	0.01		1.40		0.27	680	5	-	9.8	7.6
	0.1		1.00		0.20	720	5	-	10.2	7.7
	1		1.90	*	0.38	670	5	-	10.4	7.9
	10		4.10	*	0.28	705	5	-	9.9	7.8

[1/] Off-flavor index based on a scale of 0 (no off-flavor) to 6 (very extreme off-flavor)

[2/] A plus sign (+) indicates all test fish died.

[3/] The least significant difference at P=0.05 based on a two way analysis of variance. An asterisk (*) indicates a statistically significant change in flavor from that of the control sample.

[4/] Mean temperatures for an experiment are not listed when values were between 14.5 and 15.5°C.

Table 8. Experimental conditions and results of tests in which rainbow trout were exposed for 48 hours to various concentrations of biologically stabilized waste from paper plants located in Oregon.

Exper no.	Waste concentration (% by vol.)	Mean off-flavor index[1/2] (0-6)	LSD[3] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4] (C)	Dissolved oxygen (mg/l)	pH
E-1 Kraft process, Albany December 7, 1971 15 Judgments									
	0	0.83	0.70	0.17	652	5	-	15.2	7.5
	1.4	0.60		0.17	703	5	-	13.0	7.7
	5.6	0.87		0.19	684	5	-	10.2	7.4
	16.7	2.20	*	0.40	677	5	-	7.8	7.6
	32.8	3.43	*	0.43	597	5	-	7.4	7.2
	59.8	4.43	*	0.37	641	5	-	6.0	7.1
	100	+		-	-			6.0	7.2
E-2 Sulfite process, Lebanon January 12, 1972 15 Judgments									
	0	0.83	0.57	0.22	640	6	-	-	7.3
	1.1	0.50		0.15	471	5	-	-	7.3
	20.7	1.27		0.29	653	5	-	-	7.3
	33.5	0.83		0.19	427	5	-	-	7.3
	50.7	1.67	*	0.28	659	5	-	-	7.1
	66.5	2.33	*	0.36	664	5	-	-	6.9
E-3 Kraft process, Halsey December 18, 1971 15 Judgments									
	0	0.20	0.55	0.08	538	5	14.9	-	7.3
	9.2	1.27	*	0.27	473	5	14.8	-	7.4
	50.1	4.23	*	0.29	494	5	14.0	-	7.3
	100	5.13	*	0.28	410	4	13.1	-	7.4

Table 8. Continued

Exper. no.	Waste concentration (% by vol.)	Mean off-flavor index[1/2/] (0-6)	LSD[3/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4/] (C)	Dissolved oxygen (mg/1)	pH
E-4	Kraft process, Halsey,	January 18, 1972		15 Judgments					
	0	0.80	0.68	0.20	735	6	-	11.1	7.3
	1.3	1.07		0.18	648	5	-	10.9	7.6
	8.1	1.20		0.33	463	5	-	10.0	7.3
	20.3	2.13	*	0.29	566	5	-	10.4	7.2
	33.0	3.80	*	0.38	597	5	-	9.7	7.1
	50.0	4.47	*	0.34	604	5	-	-	7.1
	65.9	4.93	*	0.30	524	5	-	-	7.2

1/ Off-flavor index based on a scale of 0 (no off-flavor) to 6 (very extreme off-flavor).

2/ A plus sign (+) indicates all test fish died.

3/ The least significant difference at P=0.05 based on a two-way analysis of variance. An asterisk (*) indicates a statistically significant change in flavor from that of the control sample.

4/ Mean temperatures for an experiment are not listed when values were between 14.5 and 15.5°C.

Biologically stabilized kraft mill effluent (KME) was found to produce extreme high off-flavor at concentrations of 50 percent by volume and above. At concentrations between 50 and 16 percent by volume, the off-flavor indices were moderately high and ranged between 2.13 and 3.80. Interestingly enough, the effluent from the mill at Albany, which is a very old mill, produced nearly the same relationships between off-flavor and KME concentration as did effluents from the newly constructed mill at Halsey. The Halsey mill also uses about 3.5 times as much water per ton of pulp as does the Albany mill.

Shumway and Chadwick (1971) reported that untreated KME from the Albany mill caused impaired flavor in salmon at concentrations of about 1.0 to 2.0 percent by volume and above. They reported treated (biologically stabilized) KME caused no flavor impairment at concentrations of about 3.0 percent by volume. In the study reported here concentrations of stabilized KME of 1.0 to 8.1 percent by volume failed to impair flavor of trout.

Biologically stabilized effluent from an ammonia-base, sulfite mill located in Lebanon, Oregon, was found to impair the flavor of trout at concentrations of 50.7 and 66.5 percent by volume. At lower concentrations of the effluent (1.0, 20.7 and 33.7 percent by volume), no impairment of the flavor of trout was noted. The results of the tests with effluent from the sulfite mill are presented in Table 8.

Treated waste water (domestic): Experimental conditions and results of experiments with primary, secondary, and secondary chlorinated treated effluents from the Corvallis Sewage Treatment Facilities are shown in Table 9. Two experiments were conducted with primary and secondary effluents and three experiments with secondary chlorinated effluent. The effluents were provided fresh each day during the experiments.

In general, waste water that received only primary treatment was fairly toxic and impaired flavor of trout at concentrations of about 16 percent by volume and above. Secondary treated (trickling filter) waste water was non-toxic at concentrations of 100 percent by volume and produced impaired flavor in trout exposed at concentrations of 15 percent by volume and above. The addition of chlorine to the secondary treated waste water appeared to reduce the flavor-imparting capacity of the effluent. Only chlorinated secondary effluent concentrations of 20 percent by volume and above produced tainted fish. In one experiment with secondary chlorinated effluent (S-5), no flavor impairment occurred at 33 percent by volume and the fish held at higher concentrations (60 and 100 percent by volume) all died.

Discharges from waste water treatment facilities contribute tremendous volumes of treated water to lakes and streams across the nation. The data presented in this report strongly suggest that these discharges are potential hazards to the quality of the fish inhabiting the waters into which they are discharged. Secondary treatment alone does not appear to substantially reduce the flavor-imparting capacity of primary treated

Table 9. Experimental conditions and results of tests in which rainbow trout were exposed for 48 hours to various concentrations of primary and secondary treated waste from the Corvallis, Oregon Municipal Sewage Treatment Plant.

Exper. no.	Waste concentration (% by vol.)	Mean off-flavor index[1/2] (0-6)	LSD[3] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4] (C)	Dissolved oxygen (mg/1)	pH
S-1	Primary treatment	October 13, 1971	16 Judgments						
	0	0.81	0.62	0.21	1086	5	-	7.8	6.8
	3	1.12		0.39	989	5	-	9.0	7.3
	7	0.84		0.27	1037	5	-	8.5	6.8
	16	2.09	*	0.39	1052	5	-	6.2	7.1
	24	+		-	-	-	-	6.9	6.7
	34	2.44	*	0.33	1148	5	-	9.8	7.0
	50	+		-	-	-	-	-	7.0
	100	+		-	-	-	-	-	7.0
S-2	Primary treatment	December 8, 1971	15 Judgments						
	0	0.83	0.70	0.17	406	5	-	10.8	7.5
	4	1.13		0.20	411	5	-	16.4	7.5
	7	1.46		0.24	447	5	-	10.6	7.6
	25	2.63	*	0.33	485	5	-	10.8	7.5
	50	3.50	*	0.47	416	5	-	14.8	7.5
	67	4.43	*	0.37	434	5	-	12.6	7.4
	100	+		-	-	-	-	10.4	7.5
S-3	Secondary treatment	October 18, 1971	15 Judgments						
	0	0.90	0.74	0.22	962	4	-	6.0	7.2
	5	0.90		0.30	1000	5	-	14.8	7.3
	6	1.03		0.30	1072	5	-	12.0	7.4
	15	1.57		0.37	1211	5	-	9.8	7.3
	32	1.43		0.30	1054	5	-	14.2	7.3
	60	3.00	*	0.35	959	5	-	6.8	7.3
	100	4.30	*	0.37	1107	5	-	11.1	7.2

Table 9. Continued

Exper. no.	Waste concentration (% by vol.)	Mean off-flavor index[1/2] (0-6)	LSD[3] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4] (C)	Dissolved oxygen (mg/1)	pH
S-4	Secondary treatment	December 29, 1971	16 Judgments						
	0	0.78	0.64	0.18	555	6	15.1	12.0	7.2
	1	0.81		0.18	672	6	14.9	11.2	7.4
	7.4	0.62		0.15	496	6	14.8	13.2	7.5
	14.9	0.69		0.18	606	7	14.7	13.6	7.8
	32.8	2.44	*	0.27	638	6	14.6	14.6	7.2
	60.3	3.43	*	0.32	664	6	14.3	12.4	7.2
	100	4.06	*	0.33	774	7	13.7	12.2	7.1
S-5	Secondary treatment, chlorinated	October 22, 1971	16 Judgments						
	0	1.19	n.s.	0.21	1171	5	-	12.4	8.1
	1	0.63		0.23	1102	5	-	12.0	7.9
	7	0.83		0.30	1241	5	-	6.0	7.9
	10	0.94		0.25	981	4	-	13.4	8.1
	17	0.84		0.19	1215	5	-	7.6	8.0
	25	1.52		0.27	718	3	-	13.2	7.8
	33	1.72		0.34	478	2	-	13.3	8.0
	60	+		-	-	-	-	-	-
	100	+		-	-	-	-	-	-
S-6	Secondary treatment, chlorinated	November 30, 1971	15 Judgments						
	0	1.27	0.66	0.30	642	5	-	7.5	7.2
	1	0.63		0.22	561	5	-	8.0	7.2
	10	0.83		0.22	513	5	-	9.6	7.0
	17	1.83		0.38	668	5	-	7.0	6.9
	20	1.70		0.35	602	5	-	7.0	7.0
	35	2.47	*	0.34	664	5	-	8.5	6.8

Table 9. Continued

Exper. No.	Waste concentration (% by vol.)	Mean off-flavor index[1/2] (0-6)	LSD[3] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Mean temp.[4] (C)	Dissolved oxygen (mg/l)	pH
S-7	Secondary treatment, chlorinated	January 1, 1972	15 Judgments						
	0	0.67	0.68	0.20	593	8	-	11.0	7.2
	1.1	0.70		0.17	628	6	-	11.8	7.4
	19.7	1.03		0.22	764	5	-	7.6	7.3
	31.7	1.67	*	0.42	511	6	-	7.4	7.3
	49.2	2.97	*	0.47	608	5	-	11.2	7.3
	65.3	2.77	*	0.37	528	5	-	11.8	7.4

1/ Off-flavor index based on a scale of 0 (no off-flavor) to 6 (very extreme off-flavor).

2/ A plus sign (+) indicates all test fish died.

3/ The least significant difference at P=0.05 based on a two-way analysis of variance. An asterisk (*) indicates a statistically significant change in flavor from that of the control sample.

4/ Mean temperatures for an experiment are not listed when values were between 14.5 and 15.5°C.

water; chlorination appears to provide some relief, however.

In a report on potential use of waste water, Thorslund (1971) stated that "No tainting of fish flesh was noticed..." when fish were reared in large experimental ponds receiving waste water from domestic sources. Obviously, this observation disagrees with the results of the test reported here. It may well be that other methods of waste water treatment (i.e., lagoons and activated sludge) are better at reducing the tainting capacity of domestic waste water than are trickling filters.

Interaction of compounds: Many experiments were conducted in the investigation reported here to determine safe levels, or threshold concentrations, for specific organic compounds. It is rare, however, to find an effluent that contains only one compound. More commonly, effluents contain a relatively large number of different organic compounds. Information was needed to determine whether or not tainting substances acted individually on the flavor of fish or interacted in some way.

Two series of experiments were conducted to evaluate the influence of exposures to more than one tainting substance (Table 10). In the first series, two experiments were conducted in which trout were exposed to concentrations of pyridine and p-chlorophenol and combinations of both compounds. In the second series of experiments, trout were exposed to concentrations of pyridine and 2,4-dichlorophenol and combinations of both chemicals. The results of these experiments are presented in Figure 8.

In the experiments with pyridine and p-chlorophenol (Exper. M-1 and M-2), trout were exposed to pyridine concentrations of 100, 28, and 10 ppm and p-chlorophenol concentrations of 1000, 130, and 10 ppb. Trout were also exposed to the following combinations of pyridine (P) and p-chlorophenol (C): P = 18 ppm and C = 325 ppb, P = 28 ppm and C = 180 ppb, and P = 56 ppm and C = 32 ppb. As may be seen in Figure 8, exposure to the combinations of the two compounds resulted in off-flavor indices below those that might be expected. In fact, the off-flavor indices were very nearly midway between the off-flavor concentration curves determined for trout exposed to the two compounds tested (Fig. 8).

In the second series of experiments (Exper. M-3 and M-4), trout were exposed to 100 and 10 ppb of 2,4-dichlorophenol, 100 ppm of pyridine and a control. In addition, trout were exposed to 100, 10, and 1 ppb of 2,4-dichlorophenol at a pyridine concentration of 100 ppm. The combination of 100 ppm of both compounds resulted in an off-flavor index nearly the same (slightly higher) as that obtained for pyridine at 100 ppm. The additional exposure to the 100 ppb of 2,4-dichlorophenol appeared to have little or no effect on the off-flavor index. The off-flavor index of trout exposed to only pyridine, however, was considerably higher than that for trout exposed to 100 ppb of 2,4-dichlorophenol. As the concentration of 2,4-dichlorophenol decreased to 10 ppb and finally to 1 ppb in the tests with combined compounds, the off-flavor index also decreased and fell substantially below the off-flavor index for 100 ppm of pyridine.

Table 10. Experimental conditions and results of tests in which rainbow trout were exposed for 48 hours to concentrations of various chemicals and combinations of those chemicals at 15°C.

Exper. no.	Exposure concentration Chem A (ppm)	Exposure concentration Chem B (ppb)	Mean off-flavor index[1/] (0-6)	LSD[3/] .05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Dissolved oxygen (mg/l)	pH
M-1	March 31, 1971	10 Judgments,	Pyridine (A) and p-chlorophenol (B)						
	0	0	0.35	0.89	0.13	366	2	10.6	8.0
	28	0	1.75	*	0.41	373	2	11.0	-
	18	325	2.45	*	0.49	387	2	10.3	7.9
	28	130	2.40	*	0.50	365	2	10.2	8.0
	56	32	2.55	*	0.54	387	2	10.3	7.9
	0	1000	3.30	*	0.49	354	2	10.7	8.0
M-2	March 31, 1971	11 Judgments,	Pyridine (A) and p-chlorophenol (B)						
	0	0	0.32	1.05	0.15	503	3	8.8	7.8
	0	10	0.50		0.20	432	2	9.1	8.0
	10	0	1.00		0.46	478	2	9.9	7.9
	0	130	3.09	*	0.53	383	2	8.2	-
	100	0	4.64	*	0.41	450	2	10.8	7.6
M-3	June 8, 1971	9 Judgments,	Pyridine (A) and 2,4-dichlorophenol (B)						
	0	0	1.11	1.35	0.48	271	2	-	-
	100	1	2.33		0.63	314	2	-	-
	100	10	3.11	*	0.68	314	2	-	-
	100	100	4.00	*	0.54	267	2	-	-

Table 10. Continued

Exper. no.	Exposure concentration Chem A (ppm)	Exposure concentration Chem B (ppb)	Mean off-flavor index[1/] (0-6)	LSD[3/].05	Standard error of the mean	Fish per chamber grams	Fish per chamber number	Dissolved oxygen (mg/1)	pH
M-4	June 8, 1971	10 Judgments, Pyridine (A) and 2,4-dichlorophenol (B)							
	0	0	0.65	1.12	0.22	282	2	-	-
	0	10	1.30		0.28	297	2	-	-
	0	100	2.30	*	0.40	268	2	-	-
	100	0	3.85	*	0.54	288	2	-	-

1/ Off-flavor index based on a scale of 0 (no off-flavor) to 6 (very extreme off-flavor).

2/ The least significant difference at P=.05 based on a two-way analysis of variance. An asterisk (*) means a statistically significant difference in flavor from that of the control sample.

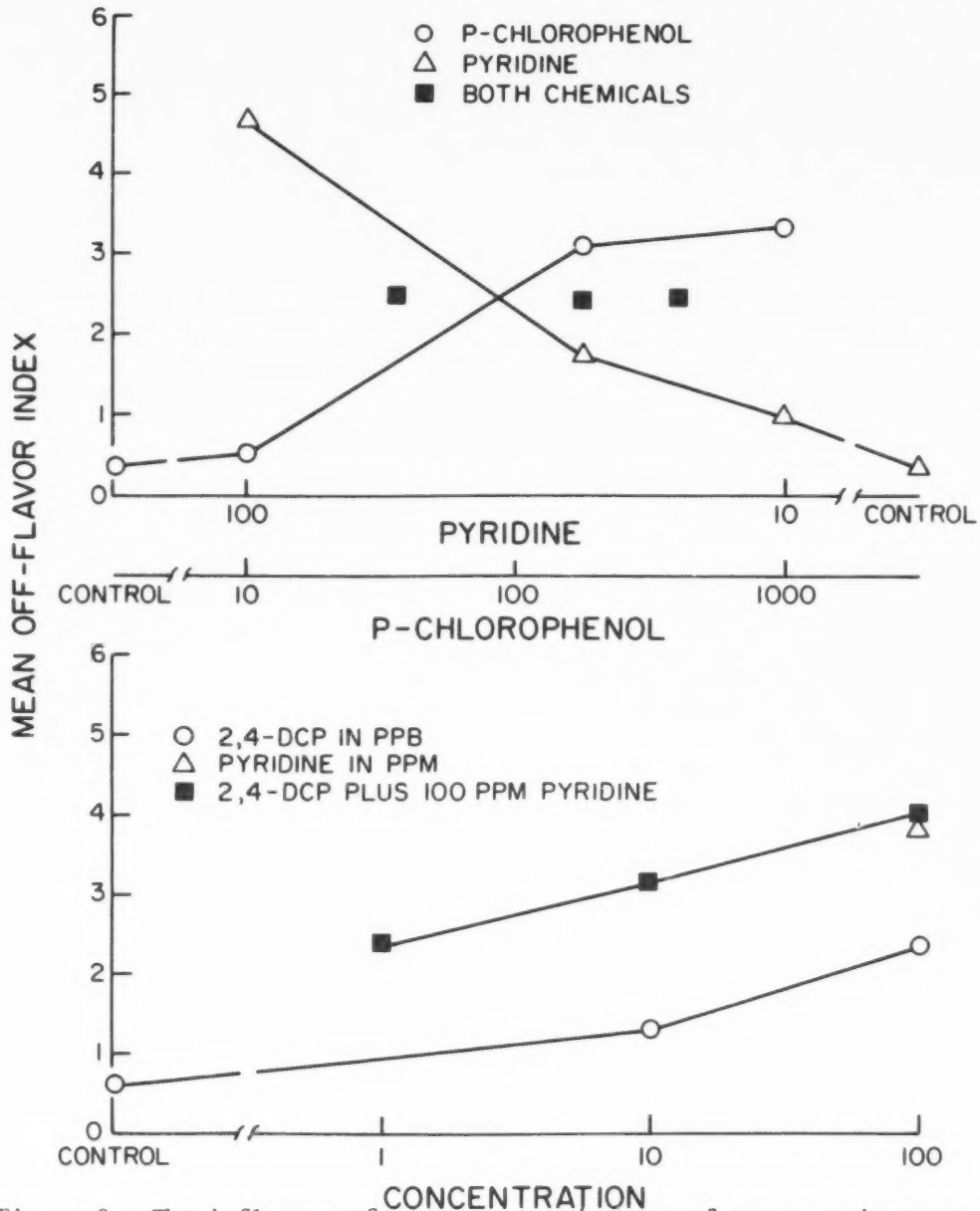

Figure 8. The influence of exposure to mixtures of two organic compounds on mean off-flavor indices. The open plots represent off-flavor indices for trout exposed to only one compound; the closed plots (squares) represent results of exposure to two compounds. The concentrations of p-chlorophenol and pyridine in the upper graph are in ppb and ppm, respectively. The presentation is based on data in Table 10.

The results of the test using combinations of two organic compounds strongly suggest that the flavor imparting capacities of organic compounds are not additive, although there does appear to be some interaction. Admittedly, the experimentation conducted in this investigation on the question of interaction of compounds is far too meager for a conclusions statement. It does appear, however, that threshold concentrations obtained for specific organic compounds should provide protection for the quality of fish if they are not exceeded, even though several compounds are involved. This may not be true, however, when compounds of very similar structure are involved. Much additional study is needed on this question.

Estimated threshold concentrations: Estimated threshold concentrations (ETC) were determined for the organic compounds and effluents tested in this study. The ETC is here defined as the highest estimated concentration of a material that will not impair the flavor of the flesh of exposed fish. In order to determine the ETC for a particular experiment, the mean off-flavor indices obtained were plotted against exposure concentration and a curve fitted by eye (See Figure 9). The curve need not pass through the control point. After the curve was fitted to the data, a mean off-flavor index was determined for the flat or independent portion of the relationship and this value was then added to the $LSD_{.05}$ value. In Figure 9 the off-flavor index for the curve was 0.58 and the $LSD_{.05}$ was 0.69. The sum of the two values is 1.27. This value was then located on the ordinate scale and a horizontal line drawn across to the eye-fitted curve. At the point of intercept, the line was extended vertically to the abscissa in the manner shown in Figure 9 (dotted line). As may be seen in Figure 9, the ETC for 2,4,6-trichlorophenol was determined as 52 ppb.

Estimated threshold concentrations were determined for the experiments with organic compounds (Tables 1 and 7) and for the effluents (Tables 8 and 9). The ETC, the highest test concentration not impairing flavor, and the lethal concentration (if determined) for the organic compounds and effluents tested are presented in Table 11 and Table 12.

The ETC for organic compounds varied from 0.4 ppb for 2,4-dichlorophenol to 95 ppm for formaldehyde. As a group the chlorinated phenols were found to have the lowest ETC values. The location and number of the chlorine ions appears important in determining the flavor-imparting capacity of this group of compounds, with the latter being most important. Another factor that may play an important role is the species of fish tested. As may be seen in Table 11, ETC values were determined for 2,4-dichlorophenol using trout, bass, and bluegill. Trout and bass produce similar ETC values (1.0 and 0.4 ppb), while the value determined using bluegills was 14 ppb. For pyridine, however, trout and bluegill gave very nearly the same ETC, 27 and 28 ppm, respectively.

The ETC for kraft process effluents ranges from 5 to 7 percent by volume; the ETC for the sulfite-base effluent was 36 percent by volume. The ranges of ETC for primary, secondary, and secondary chlorinated treated waste water were 11 to 13 percent by volume, 21 to 22 percent

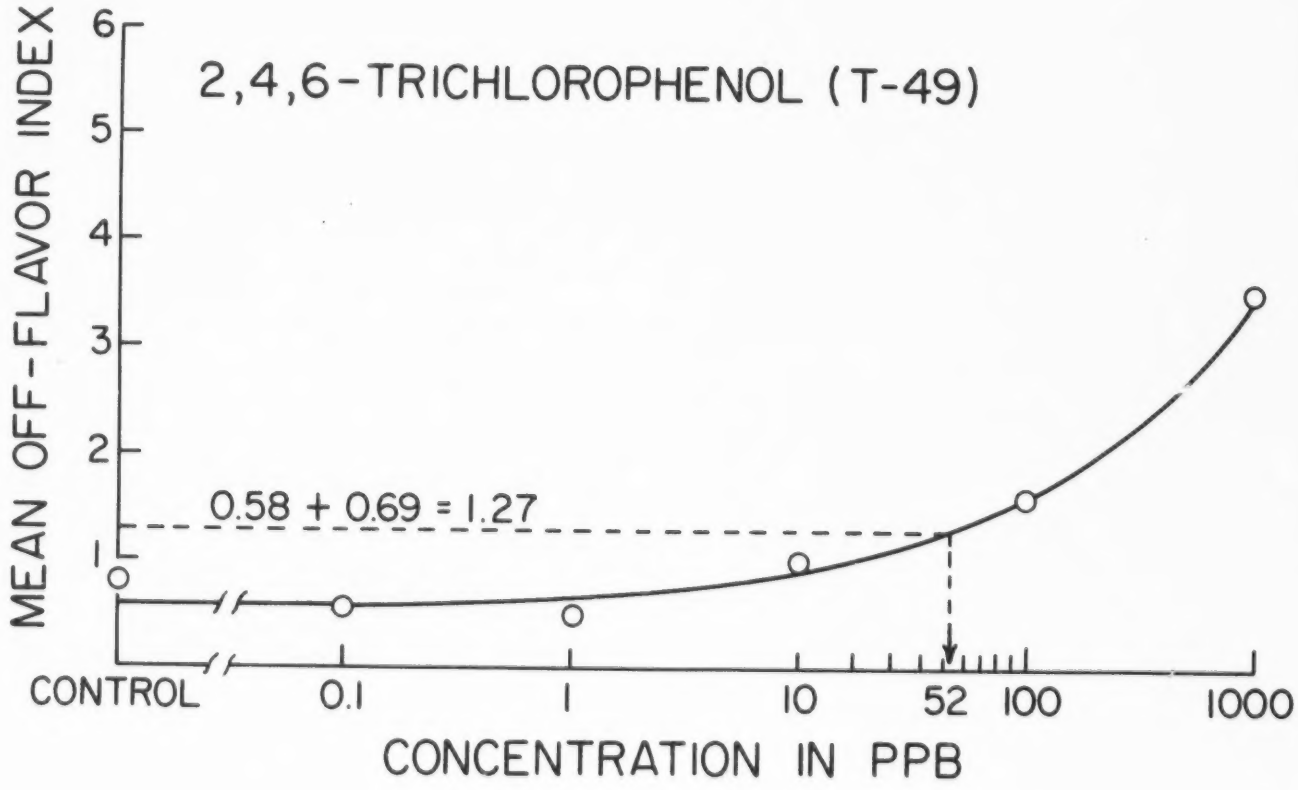

Figure 9. Example of the procedure used in determining the estimated threshold concentration (ETC). The curve was fitted to the data by size. The $LSD_{.05}$ for 2,4,6-trichlorophenol was 0.69 (Table 7, Exper. T-49).

Table 11. The highest concentrations not causing impaired flavor, lethal concentrations, and estimated threshold concentrations for the chemicals tested in this study. Data are presented for 96-hr and 48-hr tests.

Chemical tested	Test fish	Estimated threshold concentration[1]		Highest concentration not impairing flavor		Lethal concentration[2]		Test number
		ppb	ppm	ppb	ppm	ppb	ppm	
Acetone	trout	-	-	-	1,000	-	10,000	T-1
Acrylonitrile	trout	-	18	-	5.6	-	56	T-2,T-3
Amyl Acetate	trout	-	-	-	10	-	-	T-4
Aniline	trout	-	-	-	10	-	100	T-5
Benzene	trout	-	-	-	5.6	-	-	T-6
Butanethiol	trout	55	-	8	-	-	-	A-2
n-Butanol	trout	-	-	-	100	-	-	T-7
Cresol	trout	70	-	5	-	-	-	T-8,T-9
m-cresol	trout	200	-	50	-	-	-	T-10,T-11
o-cresol	trout	-	0.4	-	0.1	-	100	A-4
p-cresol	trout	120	-	50	-	-	5	T-12,T-13
Dimethylamine	trout	-	6.8	-	5.6	-	-	T-14
Ethanethiol	trout	240	-	100	-	-	-	T-15
Ethylacrylate	trout	60	-	10	-	-	-	T-20
Formaldehyde	trout	-	95	-	56	-	320	T-21,T-22,T-23
B,B'-Dichlorodiethyl ether	trout	88	-	90	-	-	91	T-24
Methylamine	trout	-	-	-	10	-	56	T-25,T-26
2, Ethyl-1-hexanol	trout	-	-	-	5.6	-	-	T-27
2-Napthol	trout	-	0.3	-	0.3	-	3.2	T-28
Sodium pentachlorophenol	trout	-	-	20	-	200	-	T-29
Phenol	trout	-	-	-	5.6	-	10	T-31,T-32
2,3-Dichlorophenol	trout	84	-	32	-	-	-	T-33
2,4-Dichlorophenol	trout	1	-	0.01	-	-	-	A-5
2,4-Dichlorophenol	bass	0.4	-	0.1	-	-	-	T-34
2,4-Dichlorophenol	bluegill	14	-	10	-	-	-	T-35

Table 11. Continued

Chemical tested	Test fish	Estimated threshold concentration[1]		Highest concentration not impairing flavor		Lethal concentration[2]		Test number
		ppb	ppm	ppb	ppm	ppb	ppm	
2,5-Dichlorophenol	Trout	23	-	10	-	-	-	T-36
2,6-Dichlorophenol	Trout	35	-	10	-	-	-	T-37
m-Chlorophenol	Trout	-	-	-	1	-	10	T-38
m-Chlorophenol	Bluegill	-	-	-	1	-	-	T-39
o-Chlorophenol	Trout	60	-	100	-	-	-	T-40,T-41
p-Chlorophenol	Trout	45	-	21	-	-	-	T-42
o-Phenylphenol	Trout	-	-	-	1	-	-	T-45,T-46
2,4,5-Trichlorophenol	Trout	-	-	320	-	1,000	-	T-48
2,4,6-Trichlorophenol	Trout	52	-	10	-	-	-	T-49
Pyridine	Trout	-	27	-	10	-	-	A-7
Pyridine	Bluegill	-	28	-	10	-	-	T-50
Pyrocatechol	Trout	-	0.8	-	0.3	-	10	T-51
Guaiacol	Trout	82	-	100	-	-	-	T-52

[1] The estimated threshold concentrations were determined by methods described earlier in the text and presented graphically in Figure 8.

[2] The lowest concentration tested at which 50 percent or more of the test fish died during the exposure period.

Table 12. Estimated threshold concentrations, highest concentrations not causing impaired flavor, and lethal concentrations for treated waste water (Corvallis) and paper process wastes.

Description of waste	Estimated threshold concentration[1/] (% by vol)	Highest concentration not causing impaired flavor (% by vol)	Lethal concentration[2/] (% by vol)	Test number
Kraft process, Albany	8	5.6	100	E-1
Kraft process Halsey	5	0	-	E-3
Kraft process Halsey	7	8.1	-	E-4
Sulfite process, Lebanon	36	33.5	-	E-2
Primary treated waste water	11	7	50	S-1
Primary treated waste water	13	7	100	S-2
Secondary treated waste water	21	32	-	S-3
Secondary treated waste water	22	14.9	-	S-4
Secondary treated, chlorinated waste water	-	33	60	S-5
Secondary treated, chlorinated waste water	20	20	-	S-6
Secondary treated, chlorinated waste water	26	19.7	-	S-7

[1/] The estimated threshold concentrations were determined by methods described earlier in the text and presented graphically in Figure 8

[2/] The lowest concentration tested at which 50 percent or more of the test fish died during the exposure period.

by volume, and 20 to 26 percent by volume, respectively. An ETC could not be determined for one experiment with secondary chlorinated effluent (Table 9, Experiment S-5), since no flavor impairment was noted at a concentration of 33 percent by volume and all test fish died at the next higher concentration of 60 percent by volume. Chlorination of treated wastewater appears to play an important role in reducing the flavor-imparting of secondary treated wastewater.

SECTION VII

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SECTION X

APPENDICES

Appendix 1. Geological Survey analysis of well water conducted February 21, 1966. Sample of water was collected after 9-months steady pumping on well.

Specific conductance (micromhos at 25°C)	259 ppm	pH	7.5
Dissolved solids (evaporated at 180°C)	165 ppm	Temperature (°C)	10
Hardness as $CaCO_3$	0	Color	0
Silica (SiO_2)	32 ppm	Sodium (Na)	8.5 ppm
Magnesium (Mg)	9.9 ppm	Nitrate (NO_3)	0.3 ppm
Potassium (K)	0.3 ppm	Fluoride (F)	0.1 ppm
Bicarbonate (HCO_3)	162 ppm	Chloride (Cl)	6.0 ppm
Carbonate (CO_3)	0	Sulfate (SO_4)	1.0
Iron (F)	0.08 ppm		

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Appendix 2. Ballot used in organoleptic evaluation of fish flesh.

Department of Food Science and Technology
Oregon State University

Name: _____

Date: _____

Test No. _____

Off-Flavor

- None
- Slight
- Moderate
- Strong
- Very Strong
- Extremely Strong
- Very Extremely Strong

Overall Desirability

- Very Desirable
- Moderately Desirable
- Slightly Desirable
- Neutral
- Slightly Undesirable
- Moderately Undesirable
- Very Undesirable

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